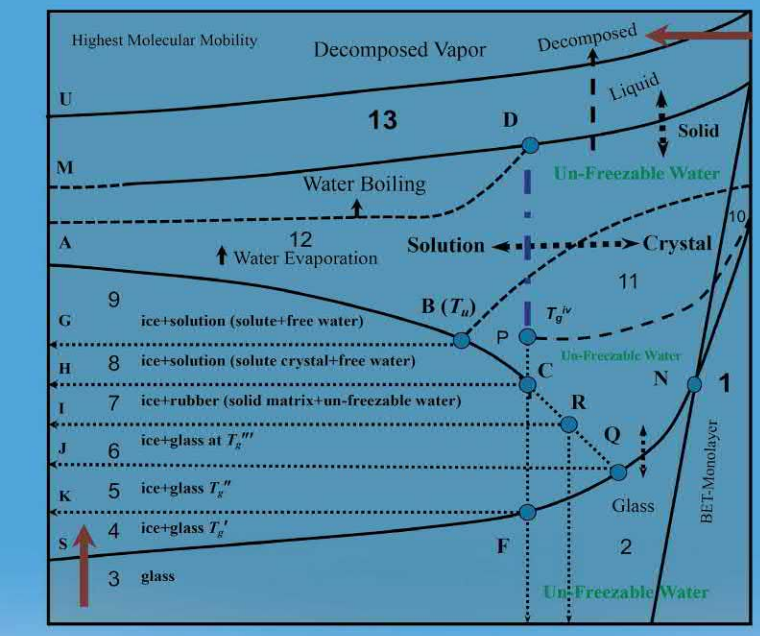

HANDBOOK OF FOOD PRESERVATION

THIRD EDITION

Edited by **Mohammad Shafiur Rahman**

CRC Press
Taylor & Francis Group

Handbook of Food Preservation

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Third Edition

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Dedication

Dedicated to the late Saleha Khatun, Sabina Akhter, Rubaba Rahman, Aiyla Rahman

Contents

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PART III Preservation Using Chemicals and Microbes

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Editor

Mohammad Shafiur Rahman, Professor at the Sultan Qaboos University, Oman, and the author/co-author of more than 400 technical articles, including 149 refereed journal papers, 137 conference papers, 78 book chapters, 36 reports, 18 popular articles, and 13 books. He is the author of the internationally acclaimed and award-wining *Food Properties Handbook*, published by CRC Press, Boca Raton, Florida, which was one of the bestsellers from CRC Press in 2002. The second edition was released with his editorship. He is also one of the editors of *Handbook of Food Process Design*, published by Wiley-Blackwell, Oxford, England, in 2012. He was invited to serve as one of the associate editors for the *Handbook of Food Science, Engineering and Technology* (2006), and one of the editors for the *Handbook of Food and Bioprocess Modeling Techniques*, published by CRC Press, Florida (2008). Since 2015, he has published five more books in the areas of food science, technology, and nutrition.

Professor Rahman has initiated the *International Journal of Food Properties* (Marcel Dekker, Inc.) and has served as its founding editor for more than 20 years. In addition, he has served in the editorial boards of more than ten international journals. In 1998, he was invited and continued to serve as a Food Science Adviser to the International Foundation for Science (IFS) in Sweden. Professor Rahman is a professional member of the New Zealand Institute of Food Science and Technology (NZ), the Institute of Food Technologists (USA), and Member of Executive Committee for International Society of Food Engineering, ISFE. He was involved in many professional activities, such as organizing international conferences, training workshops, and other related activities. In 2014, he initiated and served as the Founding Chair of the International Conference on Food Properties (ICFP) series and initiated the ICFP mentoring program for young scientists and academics. He has been invited as keynote/plenary speaker to more than ten international conferences in the food science and engineering sectors. He earned B.Sc. Eng. (Chemical) (1983) and M.Sc. Eng. (Chemical) (1984) degrees from Bangladesh University of Engineering and Technology, Dhaka, Bangladesh, an M.Sc. degree (1985) in food engineering from Leeds University, England, and a Ph.D. degree (1992) in food engineering from the University of New South Wales, Sydney, Australia.

Professor Rahman is an eminent scientist and academic in the areas of food processing and preservation. He is recognized for his significant contribution to the basic and applied knowledge of food properties related to food structure, health functionality, engineering properties, and food stability. His total SCOPUS and Google Scholar citations are 3,928 (h-index: 37) and 29,436 (h-index: 70), as of 4 July 2019, which qualifies the impact of his research in the international scientific community. Professor Rahman has received numerous awards and fellowships in recognition of research/teaching achievements, including the HortResearch Chairman's Award, the Bilateral Research Activities Program (BRAP) Award, CAMS Outstanding Researcher Award 2003, SQU Distinction in Research Award 2008, SQU Recognition for Scientific and Research Excellence (year 2017–2018), and the British Council Fellowship. In 2008, Professor Rahman ranked among the top five leading scientists and engineers of the 57 OIC Member States in the AgroSciences Discipline.

List of Contributors

Abrehem Abad
Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland and Labrador, Canada

Mohammad Aboonajmi
Department of Agrotechnology
Abouraihan Campus
University of Tehran, Iran

Jasim Ahmed
Food and Nutrition Program
Environment and Life Sciences Research Center
Kuwait Institute for Scientific Research
Safat, Kuwait

Mushtaque Ahmed
Department of Soil
Water and Agricultural Engineering
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Issa S. Al-Amri
DARIS Centre for Scientific Research and Technology Development
University of Nizwa
Nizwa, Oman

Zaher Al-Attabi
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Ismail M. Al Bulushi
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Kutaila Al-Farsi
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Nasser Al-Habsi
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Saud Musallam Al-Jufaili
Department of Marine Science and Fisheries
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Mohammed Al-Khusaibi
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Abdulaziz Y. Al-Kindi
DARIS Centre for Scientific Research and Technology Development
University of Nizwa
Nizwa, Oman

Priyatharini Ambigaipalan
Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland and Labrador, Canada

Elizabeth A. Baldwin
Citrus and Subtropical Products Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Winter Haven, Florida

Gustavo V. Barbosa-Canovas
Washington State University
Pullman, Washington

Bhesh Bhandari
School of Agriculture and Food Sciences
The University of Queensland
Brisbane, Australia

M. L. Bhavya
Department of Technology Scale-up
CSIR-Central Food Technological Research Institute
Mysuru, India

Kanishka Bhunia
Department of Biological System Engineering
Washington State University
Pullman, Washington

X. Dong Chen
Soochow University
Jiangsu, China

Titus De Silva
Food Safety Consultant
Auckland, New Zealand

Robert H. Driscoll
Food Science and Technology Program
School of Chemical Engineering and Industrial Chemistry
The University of New South Wales
Sydney, Australia

Hamideh Faridi
Department of Agrotechnology
University of Tehran
Tehran, Iran

T. V. Gamage
CSIRO Agriculture and Food
Melbourne, Australia

M. Marcela Gongora-Nieto
Washington State University
Pullman, Washington

Leon G. M. Gorris
Agrotechnological Research Institute
Wageningen, the Netherlands

Nejib Guizani
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Quazi Mohd. Imranul Haq
Department of Biological Sciences and Chemistry
College of Arts and Sciences
University of Nizwa
Nizwa, Oman

H. Umesh Hebbar
Department of Technology Scale-up
CSIR-Central Food Technological Research Institute
Mysuru, India

Thao M. Ho
Department of Food Technology
An Giang University
Vietnam University
Ho Chi Minh City, Vietnam

Isam T. Kadim
Department of Biological Sciences and Chemistry
College of Arts and Sciences
University of Nizwa
Nizwa, Oman

Afaf Kamal-Eldin
Department of Food Science
College of Food and Agriculture
United Arab Emirates University
Al-Ain, United Arab Emirates

Mehmet Murat Karaoglu
Food Engineering Department
Ataturk University
Erzurum, Turkey

Nozieana Khairuddin
Department of Basic Science and Engineering
Faculty of Agriculture and Food Science
Universiti Putra Malaysia
Bintulu, Malaysia

Mohidus Samad Khan
Department of Chemical Engineering
Bangladesh University of Engineering and Technology
Dhaka, Bangladesh

Gerard La Rooy
Round Earth Business Process Improvement
Hastings, New Zealand

Theodore P. Labuza
Department of Food Science and Nutrition
The University of Minnesota
St. Paul, Minnesota

Yubin Lan
College of Agricultural Engineering and Food Science
Shandong University of Technology
Zibo, China

Lothar Leistner
Formerly, Institute of Microbiology
Toxicology and Histology
Federal Centre for Meat Research
Kulmbach, Germany

Xuan Li
DuPont Stine-Haskell Research Center
Newark, Delaware

Marybeth Lima
Department of Biological and Agricultural Engineering
LSU AgCenter
Louisiana State University
Baton Rouge, Louisiana

Aurelio López-Malo
Universidad de las Américas
Puebla, Mexico

Ajit K. Mahapatra
Agricultural Research Station
Fort Valley State University
Fort Valley, Georgia

Msafiri Mbaga
Natural Resource Economics
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

V. K. Mishra
Institute of Sustainable Industries and Cities
Victoria University and KR Food and Bio Consultancy Services
Melbourne, Australia

Ann Mothershaw
Formerly, Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Ida Idayu Muhamad
Department of Bioprocess and Polymer Engineering
Faculty of Chemical and Energy Engineering
Universiti Teknologi Malaysia
Johor Bahru, Malaysia

Nadira Mustari
Department of Chemical Engineering
Bangladesh University of Engineering and Technology
Dhaka, Bangladesh

Kasiviswanathan Muthukumarappan
Department of Agriculture and Biosystems Engineering
South Dakota State University
Brookings, South Dakota

Amera K. Nasser
Department of Animal Production
College of Agriculture
Basrah University
Basrah, Iraq

Umezuruike Linus Opara
Faculty of AgriSciences
University of Stellenbosch
Stellenbosch, South Africa

Enrique Palou
Universidad de las Américas
Puebla, Mexico

Ronald B. Pegg
Department of Food Science and Technology
University of Georgia
Athens, Georgia

Herman W. Peppelenbos
Agrotechnological Research Institute
Wageningen, the Netherlands

Anne Perera
New Zealand Institute for Crop and Food Research
Palmerston North, New Zealand

Conrad O. Perera
School of Chemical Sciences
Food Science Program
University of Auckland
Auckland, New Zealand

Jan Pokorny
Formerly, Department of Food Chemistry and Analysis
Faculty of Food and Biochemical Technology
Institute of Chemical Technology
Prague, Czech Republic

Md Jiaur Rahman
Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland and Labrador, Canada

Md Ramim Tanver Rahman
Department of Chemistry
Faculty of Science and Engineering
Université Laval
Québec City, Québec, Canada

Rubaba Rahman
SBA Lawyers
Sydney, Australia

Hosahalli S. Ramaswamy
Department of Food Science
McGill University
Ste Anne de Bellevue, Canada

M. N. Ramesh
Formerly, Department of Food Engineering
Central Food Technological Research Institute
Mysore, India

Shyam S. Sablani
Department of Biological System Engineering
Washington State University
Pullman, Washington

Fereidoon Shahidi
Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland and Labrador, Canada

Eddy J. Smid
Agrotechnological Research Institute
Wageningen, the Netherlands

Gabriela John Swamy
Food and Biomaterials Engineering
Agricultural and Biosystems Engineering Department
South Dakota State University
Brookings, South Dakota

Barry G. Swanson
Washington State University
Pullman, Washington

P. J. Torley
School of Science
RMIT University
Victoria, Australia

T. T. Truong
School of Agriculture and Food Sciences
The University of Queensland
Brisbane, Australia

Vickie A. Vaclavik
Washington State University
Pullman, Washington

Jorge Fernando Velez-Ruiz
Departamento de Ingeniería Química y Alimentos
Universidad de las Americas
Puebla, Mexico

Humberto Vega-Mercado
Washington State University
Pullman, Washington

Mostafa Waly
Department of Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Part I

Preservation of Fresh Food Products

1 Types of Foods and Food Products

Mohammad Shafiur Rahman

CONTENTS

1.1 WHAT ARE FOODS?

Foods are materials, in a raw, processed, or formulated form, that are consumed orally by humans or animals for growth, health, satisfaction, pleasure, and satisfying social needs. Generally, there is no limitation on the amount of foods that may be consumed (as there is for a drug in the form of a dosage) [1]. However, this does not mean that we can eat as much as we want, thus we need to follow dietary guidelines. There is another category, supplements, which are also consumed as dosages mainly to prevent diseases or are beneficial to treat diseases. Chemically, foods are mainly composed of water, lipids, fats, and carbohydrates with a small proportion of minerals and organic compounds. Minerals in the form of salts and organic substances are present in foods as vitamins, emulsifiers, acids, antioxidants, pigments, polyphenols, or flavors [2].

1.2 TYPES OF FOODS

Raw foods generally originate from two major sources: the plant and animal kingdoms. Some foods hold an intermediate position, such as honey being a plant product but collected and processed by bees [3]. Foods can be classified based on their sources. For example, plant sources (e.g., fruits and vegetables) and animal sources (e.g., meat, fish, and dairy) [3]. A proper classification could be used to identify the generic characteristics found in different classes of foods. Foods can also be classified based on the degree of processing (processing aspects). In many instances, foods we consume pass through different degrees of processing actions. This affects the storage stability, safety, and convenience to consume. Processing adds value to the raw foods and generates a monetary gain. Processing is also important in achieving food security by making it available, safe, and wholesome. Figure 1.1 shows different classes of foods based on the degree of processing (tomato is considered as an example). Similarly, different meat products can be prepared from fresh meats, such as frozen, canned, modified atmosphere packed, vacuum packed, fermented, cured, and smoked meats.

1.2.1 Fresh Foods

Farmers grow or produce fresh foods and look for markets for their raw fresh products. Food processors or manufacturers utilize new technologies and processing or preservation methods in order to produce value-added products from fresh foods harvested by farmers or from their by-products.

Fresh foods have a short shelf life and need to be processed prior to perceptible evidence of undesirable changes. Processing can help foods retain their original state of physical, chemical, microbial, and sensory characteristics. The goal is to preserve the sensitive nutrients of fresh foods.

Harvested foods are items separated from the medium of immediate growth (plant, soil, or water) or meat from the animal after slaughter, eggs laid from birds, or milk from the normal secretion of mammalian glands. They are considered as fresh foods and no processing steps are taken on the products.

1.2.2 Processed Foods

Processing adds value to a food product. Food preservation or processing is an action or way to maintain foods at a desired level of properties or nature for their maximum benefits. Maintaining food properties lies at the heart of food science and technology, and it is the main purpose of food processing. Generally, a process is defined as the sequence of events and equipment systems required in producing a product. In the case of processed foods, steps are taken to improve quality in terms of safety, nutrition, desired sensory characteristics, and convenience as well as shelf life.

It is commonly considered that fresh foods contain more vitamins and functional components, and thus are expected to be the best. On the other hand, processed foods may contain added chemicals and low levels of nutrients. However, other dimensions of processed foods could be their digestibility,

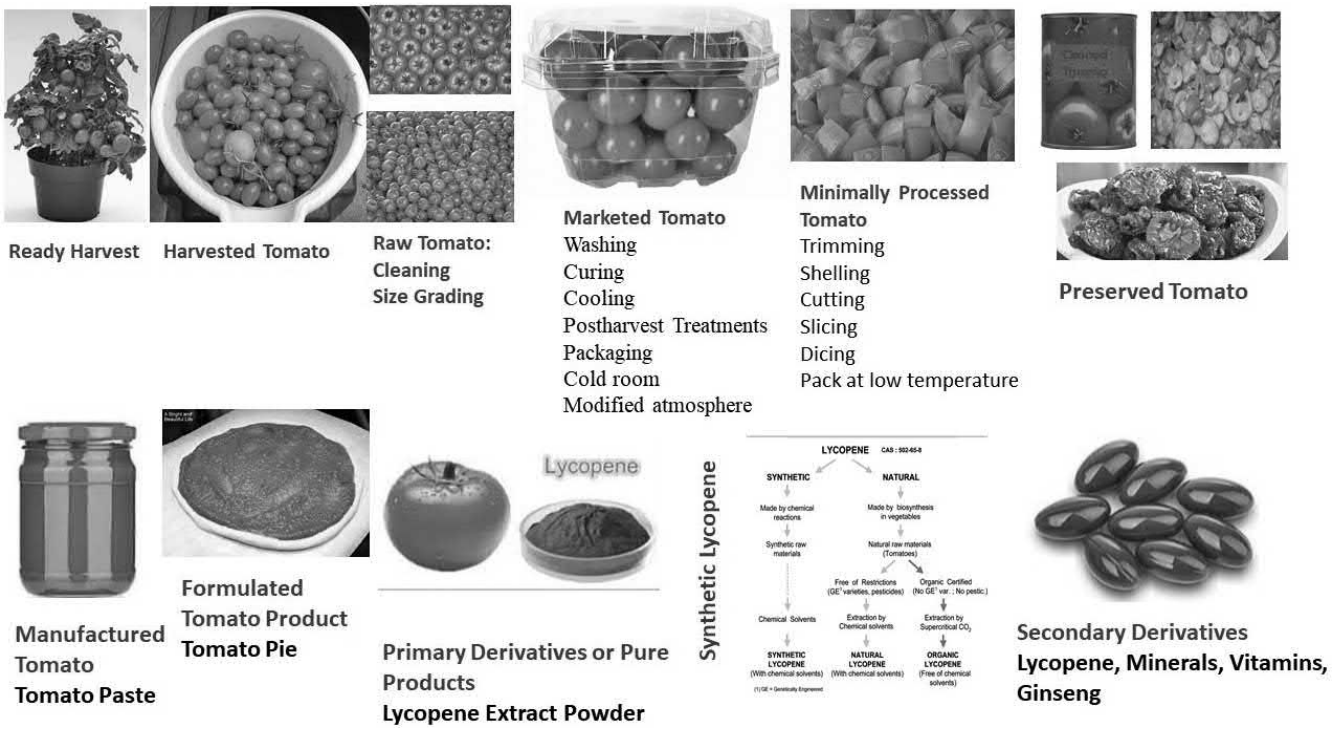

FIGURE 1.1 Different classes of food based on processing severity (tomato as an example).

nutrient bioavailability, and their function. In these cases, processing could play a role. For example, cooking at higher than 90°C or acid washing reduces pesticide residue. In addition to improved sensory perceptions and palatability, cooking improves digestibility. In the case of cassava, processing reduces cyanide (antinutrients). Cooking also releases more nutrients and bioavailability is observed. For example, in heated tomatoes and ketchup, the release of polyphenol and bioavailability of lycopene are observed.

1.2.2.1 Maintaining Original Structural Integrity

In this category, processing does not change the original structure or integrity of raw foods. The following lists foods in this category.

1.2.2.1.1 Raw Foods

Raw foods are in the earliest or primary state, after harvesting, milking, or slaughter, and have not been subjected to any treatment apart from cleaning and size grading.

1.2.2.1.2 Marketed Fresh Foods

Marketed fresh foods are harvested items retaining their complete structural integrity or state as a whole without perceptible evidence of physical, chemical, or microbiological change. The main steps in preparing marketed fresh foods are washing, curing, cooling, postharvest treatments, packaging, and storage in a cold room or modified atmosphere.

1.2.2.1.3 Minimally Processed Foods

In general, minimally processed foods have fresh-like quality and are convenient to prepare, serve, and consume (i.e., minimal steps for the consumers). In the case of minimally processed fruits and vegetables, attempts are made to keep the structural integrity without conventional food preservation techniques, such as freezing, canning, and drying (i.e., minimal processing severity). This adds to the convenience. Similar attempts are also made for meat jerky.

In the case of minimally processed fruits and vegetables, the main steps are trimming, shelling, cutting, slicing, dicing, and usually storage at low temperatures (i.e., chilled condition) to prolong quality and safety. Washing, peeling, and dicing make fruits and vegetables easy to consume.

This category has more risk than fresh or processed foods. Nowadays different product categories are appearing on the market, such as ready-to-eat meals, cook–chill and cook–freeze products, and partially cooked bread and pizza. In these products, the original structural integrity of the raw materials could not be maintained, but attempts are made to make the products appear fresh cooked or fresh baked.

1.2.2.1.4 Preserved Foods

Preserved food products are changed little during manufacturing, that is, the main preservation methods do not significantly change the individual properties of the foods. Examples include canned, frozen, and dehydrated foods.

1.2.2.2 Severity of Processing or Restructuring

In this category, processing changes the original natural structure of food at different degrees or levels. The following describes foods in this category.

1.2.2.2.1 Manufactured Foods

In manufactured food products, the raw substance usually loses its original composition by employing one or more basic methods of preservation. Examples are sausages, cured meats, jams and marmalades, and even wines.

1.2.2.2.2 Formulated Foods

Formulated food products are prepared completely based on mixing and processing of individual ingredients to make shelf-stable products, for example, cakes, biscuits, breads, and ice cream.

1.2.2.2.3 Primary Derivatives or Pure Products

Primary derivatives or pure products are obtained from the raw product through purification, for example, starch from corn or potatoes, sugar from beets, fats and oils from seeds and fish, and lycopene from tomatoes.

1.2.2.2.4 Secondary Derivatives

Secondary derivatives are derived by further steps from basic components. Examples are fat hardened by hydrogenation, cholesterol-free oil, phytochemicals and vitamins, and low molecular weight pectin by hydrolysis from the extracted pectin from fruit skins.

1.2.2.2.5 Synthetic Foods

Synthetic foods are made through microbial or chemical synthesis. Examples are vitamins, nutrients, and functional food components.

1.3 CONCLUSION

The food industry routinely develops a wide variety of food products using new technologies, new formulations, and new raw materials. It is important to understand the classes of foods for their proper production methods, safety, and legal requirements for marketing. For example, in some countries, saffron is permitted for use to color and flavor foods, but crocin extracted from saffron might not be permitted.

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2 Food Preservation: An Overview

Mohammad Shafiur Rahman

CONTENTS

2.1 INTRODUCTION

Food preservation involves the action taken to maintain foods with the desired properties or nature for as long as possible. The process is now moving from an art to highly interdisciplinary science. Food preservation methods consider the use of or combination of inactivation, inhibition, and avoidance of recontamination of foods. This chapter provides an overview of food preservation methods, emphasizing the following: (i) use of chemicals and microbes; (ii) control of water, structure, and atmosphere; (iii) use of heat and energy; and (iv) enhanced preservation by indirect approaches. The final section discusses the factors that need to be considered in order to satisfy the present and future demands of consumers and law-enforcing authorities.

Throughout most of the world, innovation, sustainability, and safety have become the focus of modern industry and economy. The United Nations World Commission on Environment and Development defines sustainable development as "meeting the needs of the present generation without compromising the ability of future generations to meet their own needs." A sustainable way of designing and developing food products appeals to consumers, provides a point of differentiation from competitors, and provides a perfect platform for a range of positive public relations activities [1]. Innovation is vital to maintain progress in technology and engineering. Food safety is now the first priority of the food production and preservation industry, incorporating innovation and sustainability. The industry can compromise with some qualities, such as color, to some extent but not with safety.

The preservation and processing of food are not as simple or straightforward as it was in the past. It is now moving from an art to a highly interdisciplinary science. A number of new preservation techniques are being developed to satisfy current demands of economic preservation and consumer satisfaction in nutritional and sensory aspects, convenience, safety, absence of chemical preservatives, price, and environmental safety. Understanding the effects on food of each preservation method has therefore become critical in all aspects. This chapter provides overviews of the new technology, identifying the changing demands of food quality, convenience, and safety.

2.2 CAUSES OF DETERIORATION

Mechanical, physical, chemical, and microbial effects are the leading causes of food deterioration and spoilage. Damage can start at the initial point by mishandling of foods during harvesting, processing, and distribution; this may ultimately lead to a reduction of shelf life. Other examples of deterioration include (i) bruising of fruits and vegetables during harvesting and postharvest handling, leading to the development of rot; (ii) tuberous and leafy vegetables lose water when kept in atmospheres with low humidity and subsequently wilt; and (iii) dried foods kept in high humidity may pick up moisture and become soggy. The four sources of microbial contaminants are soil, water, air, and animals (insects, rodents, and humans) (Table 2.1). The major causes of quality loss are shown in Table 2.2. In preservation, each factor needs to be controlled or maintained at a desired level. Foods are perishable or deteriorative by nature. The storage life of fresh foods at normal atmospheric conditions is presented in Table 2.3.

During storage and distribution, foods are exposed to a wide range of environmental conditions. Environmental factors such as pressure, temperature, humidity, oxygen, and

TABLE 2.1
Organisms That Spoil Foods

1. Microorganisms
   a. Fungi: mold and yeast
   b. Bacteria
   c. Phages
   d. Protozoa
2. Insects and mites
   a. Directly by eating (infestation)
   b. Indirectly by spreading diseases (fruit fly, housefly)
3. Rodents
   a. Directly by consuming food
   b. Indirectly by spreading disease

Source: Borgstrom [3].

light can trigger several reactions that may lead to food degradation. As consequences of these mechanisms, foods may be altered to such an extent that they are either rejected by or harmful to the consumer [2]. Damage is caused by the mishandling of foods during harvesting, processing, and distribution, which will lead to reduced shelf life of foods. Bruising of fruits and vegetables during harvesting and postharvest handling leads to the development of rot. Crushing of dried snack foods during distribution seriously affects their quality. Tuberous and leafy vegetables lose water when kept in atmospheres with low humidity and subsequently wilt. Dried foods kept in high humidity may pick up moisture and become soggy [2]. Condensation of moisture on foods or a damp atmosphere favors microbial growth, occasionally promotes the development of insects, and may indirectly lead to deterioration resulting in destructive self-heating [3]. Mechanical damage is conducive to spoilage. Bruises and wounds are such defects, and they frequently cause further chemical and microbial deterioration. Peels, skins, and shells constitute natural protection against this kind of spoilage [3]. In case of frozen foods, fluctuating temperatures are often destructive, for example, fluctuating temperatures cause recrystallization of ice cream, leading to an undesirable sandy texture. Freezer burn is a major quality defect in frozen foods that is caused by the exposure of frozen foods to fluctuating temperatures. These large fluctuations may cause a phase change by thawing or refreezing foods. Similarly, phase changes involving melting and solidifying of fats are detrimental to the quality of candies and other lipid-containing confectionary items. Shriveling occurs due to the loss of water from harvested fruit and vegetables.

Each microorganism has (i) an optimum temperature at which it grows best; (ii) a minimum temperature, at which growth no longer takes place; and (iii) a maximum temperature, above which all development is suppressed. Bacteria that grow particularly well at low temperatures are called *psychrophilic* (*cryophilic*) or low-temperature organisms. Bacteria with an optimum temperature of 20°C–45°C are *mesophilic*, and those with an optimum temperature above 45°C are *thermophilic* [3]. Microbial growth in foods results in food spoilage with the development of undesirable sensory characteristics, and in certain cases, the food may become unsafe for consumption. Microorganisms have the ability to multiply at high rates when favorable conditions are present. Prior to harvest, fruits and vegetables generally have good defense mechanisms against microbial attack, however, after separation from the plant, they can easily succumb to microbial proliferation. Similarly, meat upon slaughter is unable to resist rapidly growing microbes [2]. The pathogenicity of certain microorganisms is a major safety concern in the processing and handling of foods in that they produce chemicals in foods that are toxic to humans. Their growth on foods may also result in undesirable appearance and off-flavors. Microbial or chemical contaminants are also of concern in food deterioration. Chemicals from packaging materials may also be a source of food contamination.

Several chemical changes occur during the processing and storage of foods. These changes may cause food to deteriorate by reducing its sensory and nutritional quality. Many enzymatic reactions change the quality of foods. For example, cut fruits tend to be brown rapidly at room temperature due to the reaction of phenolase with cell constituents, as it is released in the presence of oxygen. Enzymes such as lipoxygenase, if not

TABLE 2.2
Major Quality Loss Mechanisms

Microbiological	Enzymatic	Chemical	Physical	Mechanical
Microorganism growth	Browning	Color loss	Collapse	Bruising due to vibration
Off-flavor	Color change	Flavor loss	Controlled release	Cracking
Toxin production	Off-flavor	Nonenzymatic browning	Crystallization	Damage due to pressure
		Nutrient loss	Flavor encapsulation	
		Oxidation reduction	Phase changes	
		Rancidity	Recrystallization	
			Shrinkage	
			Transport of component	

Sources: Gould [4, 7].

TABLE 2.3
Storage Life of Some Fresh Foods at Normal Atmospheric Conditions

Food	Terminology	Storage Life
Meat, fish, and milk	Perishable	1–2 days
Fruits and vegetables	Semiperishable	1–2 weeks
Root crops	Semiperishable	3–4 weeks
Grains, pulses, seeds, and nuts	Nonperishable	12 months

denatured during the blanching process, can influence food quality even at subfreezing temperatures. In addition to temperature, other environmental factors such as oxygen, water, and pH induce deleterious changes in foods that are catalyzed by enzymes [2].

The presence of unsaturated fatty acids in foods is a prime reason for the development of rancidity during storage as long as oxygen is available. While the development of off-flavors is markedly noticeable in rancid foods, the generation of free radicals during the autocatalytic process leads to other undesirable reactions, for example, loss of vitamins, alteration of color, and degradation of proteins. The presence of oxygen in the immediate vicinity of food leads to increased rates of oxidation. Similarly, water plays an important role; lipid oxidation occurs at high rates at very low water activities.

Some chemical reactions are induced by light, such as a loss of vitamins and browning of meats. Non-enzymatic browning is a major cause of quality change and degradation of the nutritional content of many foods. This type of browning reaction occurs due to the interaction between reducing sugars and amino acids, resulting in the loss of protein solubility, darkening of lightly colored dried products, and the development of bitter favors. Environmental factors such as temperature, water activity, and pH have an influence on non-enzymatic browning [2].

2.3 PURPOSE OF FOOD PRESERVATION

Preservation methods start with a full analysis and understanding of the whole food chain, including growing, harvesting, processing, packaging, and distribution; thus an integrated approach needs to be applied. Food preservation involves action taken to maintain foods with the desired properties or nature for as long as possible. It lies at the heart of food science and technology, and it is the main purpose of food processing. First, it is important to identify the properties or characteristics one wants to preserve. One property may be important for one product but detrimental to others. For example, collapse and pore formation occur during the drying of foods. This can be desirable or undesirable, depending on the desired quality of the dried product, for example, crust formation is desirable for long bowl life in the case of breakfast cereal ingredients, and quick rehydration is necessary (i.e., no crust and more open pores) for instant-soup ingredients. In another instance, the consumer expects apple juice to be clear, whereas orange juice can be cloudy.

2.3.1 Purpose of Food Preservation

An important question is, why to preserve a food? The main reasons for food preservations are to (i) overcome inappropriate planning in agriculture, (ii) produce value-added products, and (iii) provide variation in diet. The agricultural industry produces raw food materials in different sectors. Inadequate management or improper planning in agricultural production can be overcome by avoiding inappropriate areas, times, and amounts of raw food materials as well as by increasing storage life using simple methods of preservation. Value-added food products can give better-quality foods in terms of improved nutritional, functional, convenience, and sensory properties. Consumer demand for healthier and more convenient foods also affects the way that food is preserved. Eating should be pleasurable to the consumer and not be boring. People like to eat wide varieties of foods with different tastes and flavors. Variation in the diet is important, particularly in underdeveloped countries in order to reduce reliance on a specific type of grain (i.e., rice or wheat). In food preservation, the important points that need to be considered are

- What quality level is desired?
- How long to preserve?
- For whom to preserve?

After storage of a preserved food for a certain period, one or more of its quality attributes may reach an undesirable state. Quality is an elusive, ever-changing concept. In general, it is defined as the degree of fitness for use, or the condition indicated by the satisfaction level of consumers. When food has deteriorated to such an extent that it is considered unsuitable for consumption, it is said to have reached the end of its shelf life. In studying the shelf life of foods, it is important to measure the rate of change of a given quality attribute [2]. In all cases, safety is the first attribute, followed by quality. The product quality attributes can be quite varied, such as appearance, sensory, or microbial characteristics. Loss of quality is very dependent on the type of food and composition, formulation (for manufactured foods), packaging, and storage conditions [2]. Quality loss can be minimized at any stage of food harvesting, processing, distribution, and storage. When preservation fails, the consequences range broadly from the food becoming extremely hazardous to minor deterioration, such as color loss [4].

2.3.2 Period of Preservation

The second question is, how long to preserve? After storage for a certain period, one or more quality attributes of a food may reach an undesirable state. At that time, the food is considered unsuitable for consumption and is said to have reached the end of its shelf life. This level is defined by the manufacturer according to how long the product is saleable. The

"best before" date is set shorter than the shelf life with a good margin. Hence, it is usually safe and palatable to consume a product long after the best before date, provided the product has been stored at the recommended conditions. Products may be marketed with the production date "pack date" and/or a best before date. Alternative markings are "use by date" or "expiration date," which may be closer to shelf life than the best before date [5]. In studying the shelf life of foods, it is important to measure the rate of change of a given quality attribute [2]. The product quality can be defined using many factors, including appearance, yield, eating characteristics, and microbial characteristics, but ultimately the final use must provide a pleasurable experience for the consumer [6]. Loss of quality is very dependent on food type and composition, formulation (for manufactured foods), packaging, and storage conditions [4]. The various stages of food production, manufacture, storage, distribution, and sale are shown in Figure 2.1. Quality loss can be minimized at any stage and thus quality depends on the overall control of the processing chain. The major quality-loss mechanisms and consequences are shown in Table 2.1 and Figure 2.2. When preservation fails, the consequences range broadly from extremely hazardous to color loss [4]. The required length of preservation depends on the purpose. In many cases, very prolonged storage or shelf life is not needed, which simplifies both the transport and marketing

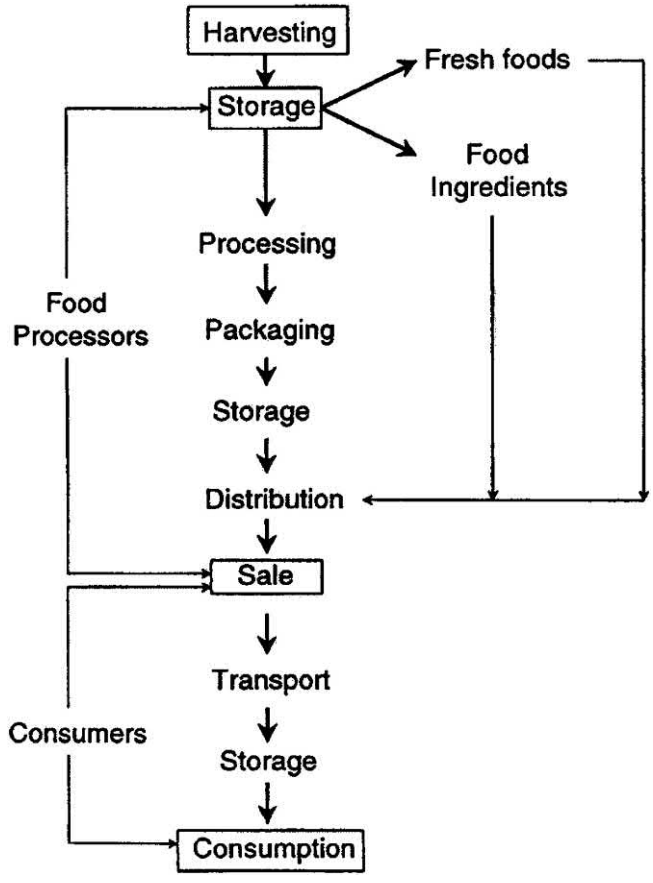

FIGURE 2.1 Various stages of food production, manufacture, storage, distribution, and sale.

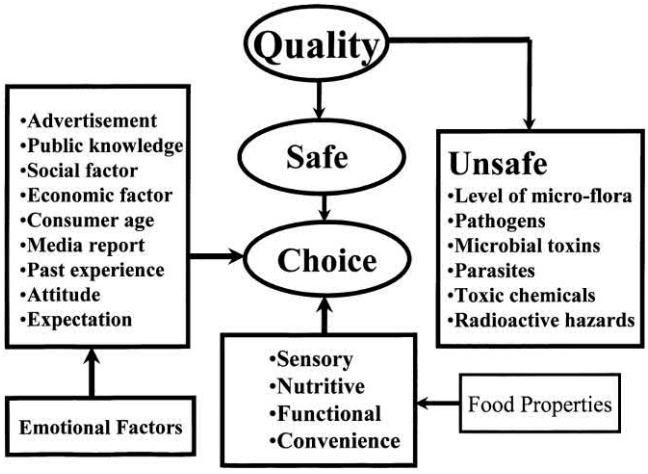

FIGURE 2.2 Factors affecting food quality, safety, and choice.

of the foodstuff. For example, prepared meals for lunch need a shelf life of only one or even half a day. In this case, there is no point in ensuring preservation of the product for weeks or months. In other cases, a very long shelf life, say, up to 3 to 5 years may be required (e.g., foods for space travelers or food storage during wars).

2.3.3 Consumers of Food Products

The third question is, for whom to preserve? It is important to know for whom the preserved food is being produced. Nutritional requirements and food restrictions apply to different population groups. Food poisoning can be fatal, especially in infants, pregnant women, the elderly, and those with depressed immune systems. The legal aspects of food preservation are different in cases of foods produced for human or animal consumption. Thus, it is necessary to consider the group for whom the products are being manufactured.

2.4 FOOD PRESERVATION METHODS

2.4.1 Gould's Classification

Based on the mode of action, the major food preservation techniques can be categorized as (i) slowing or inhibiting chemical deterioration and microbial growth; (ii) directly inactivating bacteria, yeasts, molds, or enzymes; and (iii) avoiding recontamination before and after processing [4, 7]. A number of techniques or methods from these categories are shown in Figure 2.3. Although the currently used traditional preservation procedures continue in one or more of these three ways, there have recently been great efforts to improve the quality of food products, principally in order to meet the requirements of consumers through the avoidance of extreme use of any single technique.

Preservation starts when the harvested foods are separated from the medium of immediate growth (plant, soil, or water) or meat from the animal after slaughter, or milk from the normal secretion of mammalian glands. Raw foods are those

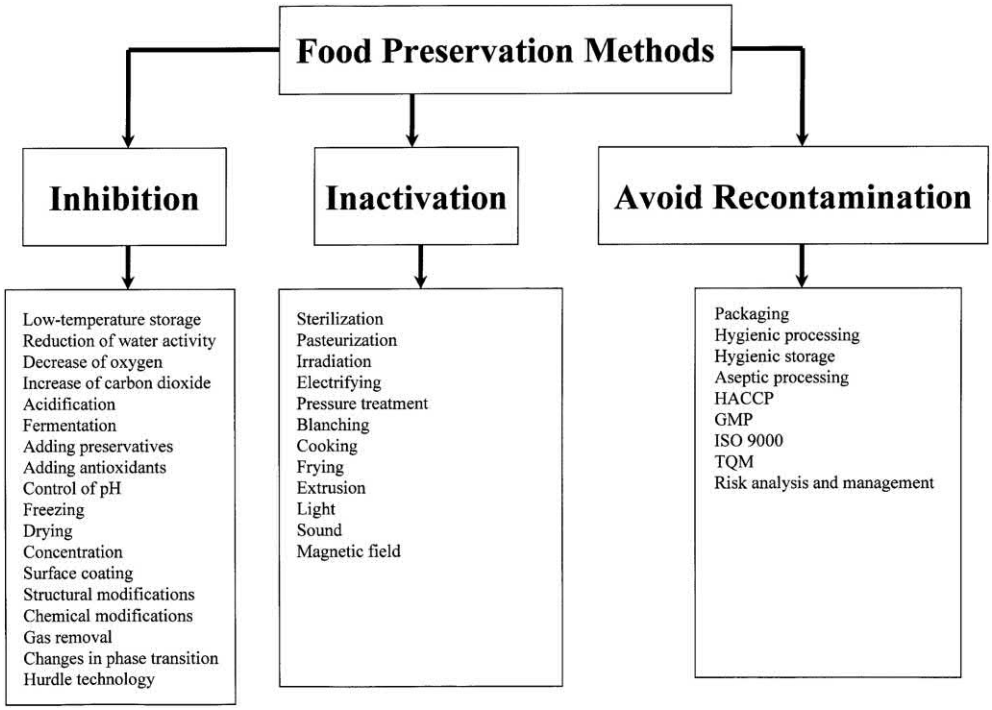

FIGURE 2.3 Major food preservation techniques [4, 7].

in the earliest or primary state after harvesting, milking or slaughter; they have not been subjected to any treatment apart from cleaning, size grading, etc., in the case of foods of plant origin. Postharvest technology is concerned with handling, preservation, and storage of harvested foods, and maintaining the original integrity, freshness, and quality. The methods of preservation depend on the origin of foods—particularly whether they are of plant or animal origin. Postharvest handling of foods of plant origin includes efficient control of environmental atmosphere (such as humidity, gas composition, and temperature), and implementing an adequate packing, storage, and transport system. Physical treatments usually used are curing, precooling, temperature treatments, cleaning, and waxing, whereas chemical treatments are disinfection, fumigation, and dipping. Meat is the edible flesh of any of a number of species of mammal or bird, both wild and domesticated. Postharvest quality is affected by slaughter conditions or stress before death.

In the case of fish, preservation methods include chilling, electrical stimulation, and decontamination methods (e.g., hot water rinsing with or without chlorination; decontamination with phosphate, hydrogen peroxide, chlorine, chlorine dioxide, and ozone; and surface treatment by organic acids). Pretreatments, such as blanching, sulfiting, and other physical and chemical pretreatments are used before applying major preservations methods. The main purpose of pretreatment is to improve product quality and process efficiency. In recent years, altering the processing strategy and/or pretreatment has gained much attention in the food industry.

The methods based on inhibition include those that rely on control of the environment (e.g., temperature control), those that result from particular methods of processing (e.g., microstructural control), and those that depend on the intrinsic properties built into particular foods (e.g., control by the adjustment of water activity or pH value [7]. The danger zone for microbial growth is considered to be between 5°C and 60°C; thus food products chilled and stored at a temperature below 5°C is one of the most popular methods of food preservation.

The steps of cleaning and sanitization are important in food preservation. Chemical disinfectants vary in their ability to kill microorganisms. Effectiveness depends on the types of microorganisms, their attachment mechanisms, and the physical characteristics of the produce. Some disinfectants are appropriate for use in direct contact washes, others only for process water, processing equipment or containers, and facilities. It is important to know disinfectants' mechanisms of action effectiveness, as well as the relevant microbial biochemistry. Several chemicals are utilized, such as chlorine, chlorine dioxide, hydrogen peroxide, ozone, peroxyacetic acid, bromine, iodine, trisodium phosphate, and quaternary ammonium compounds [8]. Although fumigants are not strictly preservatives, they are used for insect control. Methyl bromide is one of the fumigants used, but it has the potential to damage atmospheric ozone and it is being phased out. There is a need for development of new environmentally safe methods of fumigation. In reality it is very difficult to separate food preservation methods as inhibition, inactivation, and avoid recontamination, as proposed by Gould [4, 7]. For example, drying could accomplish inactivation (i.e., heat from air) as well as inhibition (i.e., low moisture). For this reason, Rahman [9] classified food preservation based on the types of applied energy and chemicals, and types of control methods.

2.4.2 Rahman's Classification

Rahman [9] classified food preservation methods into four categories: (i) preservation using chemicals and microbes; (ii) preservation by controlling water, structure, and atmosphere; (iii) preservation using heat and energy; and (iv) preservation enhanced by indirect approaches. This classification could have more rationale than classification based on mode of actions, or conventional and new methods of preservations.

2.4.2.1 Use of Chemicals and Microbes

The use of chemicals in foods is a well-known method of food preservation. Wide varieties of chemicals or additives are used in food preservation to control pH, as antimicrobes and antioxidants, and to provide food functionality as well as preservation action. Some additives are entirely synthetic (not found in nature), such as phenolic antioxidant tertiary butylhydroquinone (TBHQ), and others are extracted from natural sources, such as vitamin E. Irrespective of origin, food additives must accomplish some desired function in the food to which they are added, and they must be safe to consume under the intended conditions of use.

Many legally permitted preservatives in foods are organic acids and esters, including sulfites, nitrites, acetic acid, citric acid, lactic acid, sorbic acid, benzoic acid, sodium diacetate, sodium benzoate, methyl paraben, ethyl paraben, propyl paraben, and sodium propionate [10]. When a weak acid is dissolved in water, equilibrium is established between undissociated acid molecules and charged anions, the proportion of undissociated acid increasing with lower pH. The currently accepted theory of preservative action suggests inhibition via depression of internal pH. Undissociated acid molecules are lipophilic and pass readily through the plasma membrane by diffusion. In the cytoplasm, approximately pH 7.0, acid molecules dissociate into charged anions and protons. These cannot pass across the lipid bilayer and accumulate in cytoplasm, thus lowering pH and inhibiting metabolism [11]. There are several limitations to the value of organic acids as microbial inhibitors in foods [10]:

- They are usually ineffective when initial levels of microorganisms are high.
- Many microorganisms use organic acids as metabolizable carbon sources.
- There is inherent variability in resistance of individual strains.
- The degree of resistance may also depend on the conditions.

Nitrides and nitrates are used in many foods as preservatives and functional ingredients. These are critical components used to cure meat, and they are known to be multifunctional food additives and potent antioxidants. Many plants contain compounds that have some antimicrobial activity, collectively referred to as "green chemicals" or "biopreservatives" [12]. Interest in naturally occurring antimicrobial systems has expanded in recent years in response to consumers' requirements for fresher, more natural, and additive-free foods [7]. A range of herbs and spices are known to possess antibacterial activity because of their chemical composition. Antimicrobial agents can occur in foods of both animal and vegetable origin. Herbs and spices have been used for centuries by many cultures to improve the flavor and aroma of foods. Essential oils show antimicrobial properties, and are defined by Hargreaves as a group of odorous principles, soluble in alcohol and to a limited extent in water, consisting of a mixture of esters, aldehydes, ketones, and terpenes. They not only provide flavor to the product but also preservation activity. Scientific studies have identified the active antimicrobial agents of many herbs and spices. These include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and isothymol and thymol in oregano [13].

Rancidity is an objectionable defect in food quality. Fats, oils, or fatty foods are deemed rancid if a significant deterioration of the sensory quality is perceived, particularly aroma or flavor, but appearance and texture may also be affected. Antioxidants are used to control oxidation in foods, and these have health functionality by reducing risk of cardiovascular diseases and cancer, and slowing the aging process. The use of wood smoke to preserve foods is nearly as old as open-air drying. Although not primarily used to reduce the moisture content of food, the heat associated with the generation of smoke also gives a drying effect. Smoking has been mainly used with meat and fish. Smoking not only imparts desirable flavor and color to some foods, but also some of the compounds formed during smoking have a preservative effect (bactericidal and antioxidant).

Hydrogen ion concentration, measured as pH, is a controlling factor in regulating many chemical, biochemical, and microbiological reactions. Foods containing pH <4.5 are low-risk foods; they need less severity in heat treatment. Microorganisms require water, nutrients, appropriate temperature, and pH levels for growth. Below about pH 4.2 most other food-poisoning microorganisms are well controlled, but microorganisms such as lactic acid bacteria and many species of yeast and molds grow at pH values well below this. Many weak lipophilic organic acids act synergistically at low pH to inhibit microbial growth. Thus, propionic, sorbic, and benzoic acids are very useful food preservatives. The efficacy of acids depends to a large extent on their ability to equilibrate, in their undissociated forms, across the microbial cell membrane and in doing so, interfere with the pH gradient that is normally maintained between the inside (cytoplasm) of the cell and the food matrix surrounding it. In addition to weak lipophilic acids, other preservatives widely used in foods include esters of benzoic acid, which are effective at higher pH values than organic acids. Inorganic acids such as sulfate and nitrite are most effective at reduced pH values, like organic acids. While these preservatives are employed at parts per million (ppm) levels of hundreds to thousands, the acids used principally as acidulants are often employed at percentage levels. The following describes the three regimes of pH actions [14]:

- Strong acids do not themselves penetrate the cell membrane. These acids may exert their influence by the denaturing effect of low pH on enzymes present on the cell surface and by lowering of the cytoplasmic pH due to increased proton permeability when the pH gradient is very large.
- Weak acids, which are lipophilic and penetrate the membrane. The primary effect of such acids is to lower cytoplasmic pH and undissociated acid may have specific effects on metabolism that amplify the effects of the weak acid.
- Acid-potentiated ions, such as carbonate, sulfate, and nitrate, which are inhibitors at lower pH.

The pH affects not only microorganism growth; it also affects other components and processes, such as enzyme stability, gel formation, stability of proteins, and vitamins. Antimicrobial enzymes also have current applications and further future potential in the food industry. They play a significant role in the defense mechanisms of living organisms against infection by bacteria and fungi. Many lytic enzymes now used in the food industry to degrade unwanted polysaccharides have potential for use as novel and natural food preservatives. One such enzyme, lysozyme from hen egg whites, has been known for many years and is used against *Clostrial* spoilage in hard-cooked cheese in France [15]. When an enzyme is used, it is very important to maintain its activity for its effect on preservation. Hydrolytic antimicrobial enzymes function by degrading key structural components of the cell walls of bacteria and/or fungi, whereas antimicrobial oxidoreductases exert their effects by the generation *in situ* of reactive molecules. Fuglsang et al. [16] pointed that the potential of these enzymes in food preservation is still far from realized at present.

Antibiotics could be medical and nonmedical. Nonmedical antibiotics (such as natamycin and nisin), either produced by microbes or synthetically, inhibit microbes at very low concentrations. Organisms present in food can become resistant to antibiotics and colonize the gut of animals and man. Antibiotics used therapeutically may then become ineffective. In addition, antibiotics are used in growth enhancement and disease control in healthy animals. However, the increasing incidence of antibiotic-resistance is raising great concern and it is becoming a complicated issue.

When a chemical is used in preservation, the main question is how safe it is. There should be a risk–benefit analysis. Antimicrobial agents or preservatives are diverse in nature, but legal, toxicological, marketing, and consumer considerations have created a trend such that both the number and amount of preservatives in use are diminishing rather than increasing [16].

2.4.2.2 Control of Water, Structure, and Atmosphere

Many physical modifications are made in ingredients or foods during preservation. Such modifications can also improve the sensory, nutritional, and functional properties of foods. Changes experienced by foods during processing include glass formation, crystallization, caking, cracking, stickiness, oxidation, gelatinization, pore formation, and collapse. Through precise knowledge and understanding of such modifications, one can develop safe, high-quality foods for consumption.

Water is an important constituent of all foods. Scott in 1953 clearly identified the activity of water as a medium that is clearly correlated with the deterioration of food stability due to the growth of microorganisms and it is more important than the total amount of water as stability is concerned. This concept helps us to develop generalized rules or limits for the stability of foods using water activity. This was the main reason why food scientists started to emphasize water activity rather than water content. Since then the scientific community has explored the great significance of water activity in determining the physical characteristics, processes, shelf life, and sensory properties of foods. The minimum water activity is the limit below which a microorganism or group of microorganisms can no longer reproduce. For most foods, this is between the 0.6 and 0.7 values of the water activity range. Pathogenic bacteria cannot grow below a water activity of 0.85 to 0.86, whereas yeast and molds are more tolerant of a reduced water activity of 0.80, but usually no growth occurs below a water activity of about 0.62. The critical limits of water activity may also be shifted to higher or lower levels by other factors, such as pH, salt, antimicrobial agents, heat treatment, and temperature to some extent. Removing water, adding solutes, or a change in solute–water interactions can reduce the water activity of a food.

Drying is one of the oldest methods of food preservation, in which water activity is reduced by separating out water. Drying in earlier times was done in the sun, but today many drying methods and many types of sophisticated equipment and methods are used to dehydrate foods. Drying is a method of water removal to form final products as solids, whereas concentration means the removal of water while retaining the liquid condition. The loss of flavor, aroma, or functional compounds is the main problem with drying, in terms of quality. The cost of processing, packaging, transportation, and storage are less for dried products than canned and frozen foods. The concentration of liquid foods is mainly carried out by thermal evaporation, freeze concentration, and membrane separation. Each method has its advantages and disadvantages.

Freezing changes the physical state of a substance by changing water into ice when energy is removed in the form of cooling below freezing temperature. Usually, the temperature is further reduced to storage level at –18°C. Microbial growth is completely stopped below –18°C, and both enzymatic and nonenzymatic changes continue at much slower rates during frozen storage. There is a slow progressive change in organoleptic quality during storage. Freezing is more popular than drying due to its ability to retain relatively fresh-like qualities in the food.

Foods can be considered very stable in the glassy state since below glass temperature, compounds involved in deterioration reactions take many months or even years to diffuse

over molecular distances and approach each other to react. The hypothesis has recently been stated that this transition greatly influences food stability, as the water in the concentrated phase becomes kinetically immobilized and therefore does not support or participate in reactions. The formation of a glassy state results in a significant arrest of translational molecular motion, and chemical reactions become very slow. Many attempts are being made to relate the glass concept to physicochemical changes in foods.

Edible coatings serve many purposes in food systems. Coatings are used to improve appearance or texture and reduce water loss. Examples include the waxing of apples and oranges to add gloss, edible natural polymeric coating of frozen fish to add gloss and reduce shrinkage due to water loss, or coating of candies to reduce stickiness. Other surface treatments for foods include the application of antioxidants, acidulants (or other pH-control agents), fungicides, preservatives, and mineral salts. The formulation of edible coatings depends on the purpose and type of products. Encapsulation has been used by the food industry for more than 60 years. In a broad sense, encapsulation technology in food processing includes the coating of minute particles of ingredients (e.g., acidulants, fats, and flavors) as well as whole ingredients (e.g., raisins, nuts, and confectionery products), which may be accomplished by microencapsulation and macrocoating techniques.

Gums and gels, such as casein, guar gum, agar, carrageenan, and pectin, are also used in food products to provide the desired structure and functionality to the products. These are extremely important for the textural attributes, such as creaminess and oiliness of formulated products, and oral perception of fat-mimicking foods.

Packaging techniques based on altered gas compositions have a long history. The respiratory activity of the various plant products generates a low-oxygen and high-carbon dioxide atmosphere, which retarded the ripening of fruit. Modified atmosphere packaging is a preservation technique that may further minimize the physiological and microbial decay of perishable produce by keeping them in an atmosphere that is different from the normal composition of air. The gas composition and method of this technique depends on the type of produce and purpose. There are different ways of maintaining a modified atmosphere. In modified atmosphere packaging (termed "passive atmosphere") the gas composition within the package is not monitored or adjusted. In "controlled atmosphere packaging" the altered gas composition inside the packaging is monitored and maintained at a preset level by means of scrubbers and the inlet of gases. Active packaging can provide a solution by adding materials that absorb or release a specific compound in the gas phase. Compounds that can be absorbed are carbon dioxide, oxygen, water vapor, ethylene, or volatiles that influence taste and aroma. Vacuum and modified-humidity packaging contain a changed atmosphere around the product. Although this technique was initially developed to extend the shelf life of fresh products, it is now extended to minimally processed foods from plant and animal sources.

2.4.3 Use of Heat and Energy

2.4.3.1 Heat

Earlier, mostly heat was used for inactivation. Thermal inactivation is still the most widely used process for food preservation. The advantages of using heat for food preservation are (i) heat is safe and chemical-free; (ii) heat provides tender-cooked flavors and taste; (iii) most spoilage microorganisms are heat-labile; and (iv) thermally processed foods, when packed in sterile containers, have a very long shelf life. The main disadvantages of using heat are overcooking, which may lead to textural disintegration and an undesirable cooked flavor, and nutritional deterioration results from high-temperature processing. Heat treatment processes include mainly pasteurization, sterilization, cooking, extrusion, and frying. Currently more electrotechnologies are being used and this will expand in the future.

Considering the severity of the heat treatment process, mild processing technologies, such as high-pressure processing, ultrasounds, pulsed electric fields, ultraviolet (UV) light, high-intensity pulsed light, magnetic field, and atmospheric cold plasma can serve as useful alternatives to commercial sterilization and pasteurization, and can be used to destroy foodborne pathogens while retaining nutritional and sensorial properties. Each mild technology has a specific mode of microbial inactivation and their knowledge is of foremost importance in applying and designing quality and safe foods [17].

2.4.3.2 High Pressure and Ultrasound

High-quality fresh foods are very popular; consequently, there is demand for less extreme treatments and/or fewer additives. High-pressure hydrostatic technology gained attention for its novelty and nonthermal preservation effect. Studies examining the effects of high pressure on food date back to the end of the 19th century, but renewed research and commercialization efforts worldwide could soon bring high-pressure-treated foods back to several markets. The basis of high hydrostatic pressure is the Le Chatelier principle, according to which any reaction, conformational change, or phase transition that is accompanied by a decrease in volume will be favored at high pressures, while reactions involving an increase in volume will be inhibited. Predictions of the effects of high-pressure treatments on foods are difficult to generalize due to the complexity of foods and the different changes and reactions that can occur. However, a tremendous amount of information is being developed on microorganisms, chemical, biochemical and enzymatic reactions, development of functional and sensory properties, gel formation, gelatinization, and the freezing process.

Ultrasound is sound energy with a frequency range that covers the region from the upper limit of human hearing, which is generally considered to be 20 kHz. The two applications of ultrasound in foods are (i) characterizing a food material or process, such as estimation of chemical composition, measurements of physical properties, nondestructive testing of quality attributes, and monitoring food processing; and (ii)

direct use in food preservation or processing. The beneficial or deteriorative use of ultrasound depends on its chemical, mechanical, or physical effects on the process or products.

2.4.3.3 Electricity

Many different forms of electrical energy are used in food preservation, e.g., ohmic heating, microwave heating, low electric field stimulation, high-voltage arc discharge, and high-intensity pulsed electric field. *Ohmic heating* is one of the earliest forms of electricity applied to food pasteurization. This method relies on the heat generated in food products as a result of electrical resistance when an electric current is passed through them. In conventional heating methods, heat travels from a heated surface to the product interior by means of both convection and conduction, which is time-consuming, especially with longer convection or conduction paths. Electroresistive or ohmic heating is volumetric by nature, and thus has potential to reduce overprocessing. It provides rapid and even or uniform heating, providing less thermal damage and increased energy efficiency. *Microwave heating* has been extensively applied in everyday households and the food industry, but the low penetration depth of microwaves into solid food causes thermal nonuniformity. *Low electric field* stimulation has been explored as a method of bacterial control of meat. The mechanism of microbial inactivation by electric field was first proposed by Pareilleux and Sicard [18]. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field; permeation then causes swelling and the eventual rupture of the cell membrane. The reversible or irreversible rupture (or electroporation) of a cell wall membrane depends on factors such as intensity of the electric field, number of pulses, and duration of pulses. This new electroheating could be used to develop new products with diversified functionality.

2.4.3.4 Irradiation

Ionization radiation interacts with an irradiated material by transferring energy to electrons and ionizing molecules by creating positive and negative ions. The irradiation process involves exposing the foods, either prepackaged or in bulk, to a predetermined level of ionization radiation. The radiation effects on biological materials are direct and indirect. In direct action, the chemical events occur as a result of energy deposition by the radiation in the target molecule, and the indirect effects occur as a consequence of reactive diffusible free radicals formed from the radiolysis of water, such as the hydroxyl radical (OH^-), a hydrated electron (e_{aq}^-), a hydrogen atom, hydrogen peroxide, and hydrogen. Hydrogen peroxide is a strong oxidizing agent and a poison to biological systems, whereas the hydroxyl radical is a strong oxidizing agent and the hydrogen radical is a strong reducing agent. Irradiation has wide scope in food disinfection, shelf-life extension, decontamination, and product quality improvement. Although it has high potential, there are concerns about legal aspects and safety issues, and consumer attitudes toward this technology.

UV radiation has long been known to be the major factor in the antibacterial action of sunlight. It is mainly used in sterilizing air and thin liquid films due to its low penetration depth. When used at high dosage there is a marked tendency toward flavor and odor deterioration before satisfactory sterilization is achieved. UV irradiation is safe, environmentally friendly, and more cost-effective to install and operate than conventional chlorination. Visible light and photoreactivation are also used in food processing. If microorganisms are treated with dyes, they may become sensitive to damage by visible light. This effect is known as photoreactivation. Some food ingredients could induce the same reaction. Such dyes are said to possess photodynamic action. White and UV light are also used to inactivate bacteria, fungi, spores, viruses, protozoa, and cysts. Pulsed light is a sterilization method in applications where light can access all the important volumes and surfaces. Examples include packaging materials, surfaces, transmissive materials (such as air, water, and many solutions), and many pharmaceuticals or medical products. The white light pulse is generated by electrically ionizing a xenon-gas-filled lamp for a few hundred millionths of a second with a high-power, high-voltage pulse.

In many cases, it would be very difficult to make a clear distinction between inhibition and inactivation. Take, for example, preservation by drying and freezing. Although the main purpose of freezing and drying is to control the growth of microorganisms, there is also some destruction of microorganisms. Freezing causes the apparent death of 10% to 60% of the viable microbial population and this gradually increases during storage.

2.4.3.5 Magnetic Field

Magnetism is a phenomenon by which materials exert an attractive or repulsive force on other materials. The origin of magnetism lies in the orbital and spin motions of electrons, and electron interactions with each other. Magnetic fields have potential in pasteurization, sterilization, and enhancing other factors beneficial to processing in food preservation.

2.4.4 Food Preservation Enhanced by Indirect Approaches

In addition to the direct approach, other measures such as packaging and quality management tools need to be implemented in the preservation process to avoid contamination or recontamination. Although these measures are not preservation techniques, they play an important role in producing high-quality, safe food. With respect to the procedures that restrict the access of microorganisms to foods, the employment of aseptic packaging techniques for thermally processed foods has expanded greatly in recent years both in the numbers of applications and in the numbers of alternative techniques that are commonly available [7].

From skins, leaves, and bark, tremendous progress has been made in the development of diversified packaging materials and in the packaging equipment. Packaging performs three main functions. The first is to control the local environmental conditions to enhance storage life. The second is the display, i.e., preservation of the product in an attractive

manner to the potential buyer. The third function is to protect the product during transit to the consumer. The new concept of active or life packaging materials allows (i) one-way transfer of gases away from the product or the absorption of gases detrimental to the product; (ii) antimicrobial in packaging; (iii) release of preservatives from controlled-release surfaces; (iv) oxygen scavengers; (v) carbon dioxide generators; (vi) absorbers or scavengers of odors; and (vii) absorption of selected wavelengths of light. These systems have capabilities for controlled automatic switching. Another concept of edible or biodegradable packaging has also been evolved for environmental reasons. Processing and packaging can be integrated to improve efficiency.

Food safety has been of concern since the Middle Ages, and regulatory measures have been enforced to prevent the sale of adulterated or contaminated food. Food safety is now the highest priority. Recently the concepts of hazard analysis and critical control point (HACCP), ISO 9000, good manufacturing practices (GMP), standard operating procedures (SOP), hazard and operability studies (HAZOP), and total quality management (TQM) have gained attention. HACCP is a state-of-the-art prevention approach to safe food production based on prevention and documentation and is thus cost-effective. It is a proactive approach based on science. Most of the food industry around the globe is now targeting the implementation of HACCP programs for their processes to ensure safety. HACCP is a scientific, rational, and systematic approach to identification, assessment, and control of hazards during production, processing, manufacturing, preparation, and use of food to ensure that it is safe when consumed. This concept is based on the application of prevention and documentation. The HACCP system provides a preventive and thus a cost-effective approach to food safety. It is important to understand the concept of safety and quality first before planning to implement HACCP in the branch of the food industry or the products being targeted. The concepts of HACCP were initiated in the 1950s by the National Aeronautics and Space Administration (NASA) and Natick Laboratories for use in aerospace manufacturing. This rational approach to process control for food products was jointly developed by the Pillsbury Company, NASA, and US Army Natick Laboratories in 1971 in order to apply a zero-defects program to the food process industry [19]. The World Health Organization (WHO) has recognized the importance of the HACCP system for the prevention of food-borne diseases for over 20 years and has played an important role in its development and promotion. One of the highlights in the history of the HACCP system was in 1993 when the Codex guidelines for the application of the HACCP system were adopted by the FAO/WHO Codex Alimentarius Commission, requiring them for international trade.

ISO 9000 is the generic standard that specifies minimum requirements to be fulfilled by organizations in order to meet a customer's needs. It does not specifically address the issue of food safety, but it addresses the need to identify and comply with regulatory requirements that are applicable to the product and/or process. ISO 9000 and HACCP techniques are complementary. HACCP techniques should therefore be used as a tool to support the quality management system ISO 9000.

In order to meet the requirements of GMP, regulatory bodies provided well-defined guidelines for food-processing operations. GMP could be considered as the building blocks and cornerstones of HACCP. TQM is a management philosophy that seeks continuous improvement in the quality of performance of all processes, products, and services of an organization. HAZOP is a systematic structured approach to questioning the sequential stages of a proposed operation in order to optimize the efficiency and the management of risk. The Food Regulatory Authorities around the world are now very active in implementing these tools in the food industry. A quality management system does not guarantee food safety unless the hazards are identified and controlled.

Recently, the concept of *hurdle technology*, or combined methods of preservation, has gained attention. The microbial stability and safety of most traditional and novel foods is based on a combination of several preservative factors (called hurdles), which microorganisms present in the food are unable to overcome. This is illustrated by the so-called hurdle effect, first introduced by Leistner and his coworkers. He acknowledged that the hurdle concept only illustrates the well-known fact that complex interactions of temperature, water activity, pH, and redox potential are significant for the microbial stability of foods. With respect to procedures that slow or prevent the growth of microorganisms in foods, major successes have been seen and new applications are steadily being made in the use of combination preservation techniques or hurdle technology. This has been supported by a greatly improved understanding of the principles underlying the stability and safety of an enormous number of combination-preserved foods that are traditional and indigenous to different parts of the world. Modified atmosphere packaging has grown rapidly, particularly for the extension of the high-quality shelf life of certain chill-stored foods.

Applications of modern biotechnology with genetic modification will play a more important role in the future for more value-added products, and ease and make efficient the methods of preservation. Biotechnology is a general term for several techniques that use living organisms to make or modify products for a specific purpose. The techniques of biotechnology offer opportunities to address consumer issues of food quality and environmental safety. Biotechnology can be used to make fruit more flavorful; improve nutritional and functional quality of fruits, vegetables, grains, and muscle foods; grow foods in a wider climate zone; and grow foods in a more environmentally benign fashion [20]. The biggest application of biotechnology will be rapid and sensitive diagnostic kits for the detection of pathogens and unwanted xenobiotic compounds in foods. Another application of biotechnology will be on-package sensors that could indicate when a food is spoiled or when a pathogen or its toxic by-product is present at some level of concern [21].

The major driving forces in development and modification of food processing are the desire to reduce the extent of

processing, i.e., the demand for *lightly processed* or *fresh-like* organic and natural foods; the desire to maximize automation, control, and efficiency; the desire to minimize cost; and the need to respond to ever-more strict regulations concerning the environmental impact of various processes [22]. Nonthermal preservation technology is being used to maintain nutrition and quality. There is a tendency to reduce the intake of animal products and to consume more cereal and cereal-based products, fruits, and vegetables. Other technologies being developed to meet the consumer desire for minimally processed foods is the shift from heat treatment for pasteurization and cooking to the use of electromagnetic waves, such as electron-beam and gamma radiation and microwave radiation. Microwave applications are easily accepted by the consumer but are used in few processing applications by the food industry. One of the major problems of this technology is that to permit appropriate textures for the products, the intensity must be high enough to kill all pathogens despite their rapid time–temperature history [21]. Other potential electromagnetic processing techniques that can be used to minimize adverse heat changes due to cooking include pulsed light at high intensity, pulsed magnetic fields, direct current in a particulate stream (ohmic heating), pulsed electric discharge, and radio frequencies such as infrared. Food processing is very energy-intensive, and reducing energy use by using efficient electrotechnology can increase profit as well as reduce environmental impact. In many cases, fast or rapid heating by electrotechnology may not provide enough time to develop desired textures and flavors.

Food habits have been a very important component of human society since its inception. Changing trends and lifestyles demand specific attributes. These include convenience in preparation and consumption, changing taste preferences, attitudes and perceptions about diet and health, more nutritional and functional advances in technology that influence food quality and availability, economic factors, ethnic and geographic regional factors, age, and suitability and convenience for lifestyle [20]. Eating away from home no longer means just sitting down in a restaurant. It can be done while sitting in a car, a train, a park bench, or at an office desk. New types of fast foods are emerging to meet the demand, and safety and innovation are needed. Taste, nutrition, and convenience are the driving forces in today's market. There is also great emphasis on simple meal preparation at home, especially with microwave cooking.

Antinutritional factors in many raw materials need to be considered, and adequate pretreatments should be used before major preservation steps. It is important to reduce pesticide residues in the final products and these must be used as little as possible. There is increasing utilization of integrated pest management (IPM) as a part of growing and processing. Usually, pesticide residues decrease, often dramatically, during processing and preparation. The process of washing and peeling fruits and vegetables generally results in a significant decline of pesticides detected in the food. This is especially true for pesticide residues that are found only on the surface of the commodity [23]. The amount of residue depends largely on the pesticide, the commodity, and the process used. Exceptions may also occur when processing caused degradation of the chemical, creating a chemical that is more toxic than the parent chemical. A stewardship program is used for control of pesticide residues. There is great consumer concern regarding potentially harmful chemicals in the food supply, such as hydrocarbons, dioxin, and heavy metals.

The factors that should be considered before selecting a preservation process are (i) desired quality of the products, (ii) economics of the process, and (iii) environmental impact of the methods. Food industry waste is now also of concern to law enforcement authorities and consumers. Food waste is not only an economic loss but also has an impact on the environment. It is important to make every effort to minimize waste, to set up effective recycling systems, and to implement suitable systems for value-added products. The ultimate success of the food industry lies in the timely adoption and efficient implementation of emerging new technologies to satisfy the present and future demands of the consumer.

2.5 CONCLUSION

Continuous innovation and progress are being made in the development of preservation methods using new technologies and their combinations. Commercial successes of these new technologies are limited due to their cost, complexity, and established evidence of safety. The applications of smart food preservation methods need to be implemented to achieve food security, safety, and sustainability. In addition, consumers are now very concerned about the use of chemicals, sustainability, and environmental safety. Future food preservation methods need to address all issues rather than only considering the technological progress.

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3 Methods of Peeling Fruits and Vegetables

Xuan Li

CONTENTS

3.1 INTRODUCTION

Peeling is a common unit process for many fruits and vegetables to produce fresh-cut, minimally processed, and canned food products. The peeling process intends to remove the inedible or undesirable layer of rind or skin from raw produce. Through the peeling process, peeled products are prepared to meet high quality and safety requirements for human consumption or for other forms of subsequent processing, such as dicing, cutting, and canning. In food production lines, the peeling operation represents an important preparatory step, and it is typically performed at the initial stage [1]. After peeling, the appearance, texture, flavor, or nutrient values of the peeled product may be altered from raw materials. Hence, the peeling process can significantly contribute to changes in the storability, palatability, digestibility, and bioavailability of final food products [2].

Historically, manual peeling was the most widely adopted approach for thin skin removal and nowadays is still in practice for certain high-value fruits, such as mangoes [3]. The simplistic goal of manual peeling is to remove peels with as little loss as possible of the usable food materials [4]. Industrial peeling methods that are adaptable to large-scale process operations were successfully developed around the 1940s to 1950s to reduce labor, time, and waste involved in the peeling process [1]. Hot lye peeling and steam peeling are two widely adopted methods suitable for peeling a broad variety of fruits and vegetables, such as potatoes, tomatoes, citrus, sweet potatoes, carrots, pumpkins, pears, peaches, and apples [3]. In recent years, the reduction of energy and water consumption involved in the peeling process has become an important consideration due to the dwindling water supply and ever-tightening environment regulations [1, 4]. Producing high quality peeled products in a cost-effective manner using less water and energy drives the sustainable development of novel peeling technologies.

3.2 PEELING METHODS: CONVENTIONAL

As a unit operation, peeling can be realized by means of mechanical, chemical, or thermal treatments as well as any of their combinations. Common industrial peeling methods and emerging peeling techniques are described in the following sections.

3.2.1 Mechanical Peeling

Mechanical peeling utilizes knives, blades, or abrasive devices to remove the undesirable part directly from raw food materials [5, 6]. Thereafter, skin residuals are washed away using water. Since it is mostly performed in dry form at ambient temperature, mechanical peeling causes the least injury to the freshness and nutritional values of peeled products [3]. As compared to other chemical- and thermal-based methods, mechanical peeling operates using less energy and with lower capital costs, and has the minimum negative environmental impact. But relatively low throughput and high peeling losses (23–30%) limit its application to some high-value fruits and a few root vegetables that are difficult to peel using other means. Common mechanically peeled products include citrus

fruits, pears, pineapples, bulb onions, carrots, squashes, cassavas, and other tubers [7, 8].

Mechanical peeling can be classified as abrasive peeling and non-abrasive knife peeling [9]. In abrasive peeling, food materials, such as carrots and potatoes, are treated in a batch or continuous process equipped with abrasive elements, such as stiff brushes, carborundum rollers, and revolving bowls or drums with abrasive surfaces along the inner wall [10]. The abrasive element removes the outer skins through the shear stress developed at the peel–flesh interface. In the non-abrasive peeling, stationary knives or razor-like blades are used to remove tough-skinned product by pressing the cutting tool against the surface of the rotating materials [3, 4]. Abrasive peeling and non-abrasive knife peeling can be integrated into one single process, which is usually used for tough-skinned vegetables like parsnips, swedes, and turnips. Such a design allows products to be treated in sequence by the abrasive devices and knife tools with adjustable rotating speed and controlled depth of knife penetration, yielding a finished product with a much cleaner cutting surface. Cutting tools and abrasive elements can be custom-designed to match the geometrical characteristics of the food products and the mechanical properties of the skin to be peeled. Variability in product shapes and sizes, the difference in skin thickness, texture, and strength of skin adhesion to the flesh are the key considerations in the design of mechanical peeling devices.

3.2.2 Lye Peeling

Lye peeling, also known as caustic peeling, is a chemical method used for peel removal of thin-skinned products, where raw food materials are exposed to a heated solution containing caustic chemicals (most commonly, sodium hydroxide or potassium hydroxide) that can dissolve the skin. After treatment with lye, the loosened skins can be removed either by high-pressure water sprays or through abrasive peel eliminators, usually a perforated rotary drum or a mechanical pinch roller [2].

Lye peeling can be further divided into two categories: wet lye peeling and dry lye (dry-caustic) peeling. The wet lye peeling is carried out by immersing products into a concentrated lye solution of 8–25% at an elevated temperature from 60 to 100°C for a short residence time (15–30 s) [3]. Fundamentally, the presence of hydroxyl (OH^-) group in lye solution cleaves the α (1–4) bonds of individual galacturonic acid units in polysaccharides of skins [11]. As it moves further into the product's inner tissues through the diffusion mechanism, the lye solution dissolves the pectic and hemicellulosic materials and weakens the network of cellulose microfibrils, thus loosening the skin [12, 13]. In some cases, different chemical additives, such as surfactants or wetting agents, are added to the lye bath to improve peeling efficiency or to reduce lye concentration while maintaining the peeling effectiveness [1, 14].

Dry-caustic peeling is a modification of the wet lye peeling method. Instead of soaking products in a hot lye bath, a lye solution is sprayed onto product surfaces under a high-temperature environment, where thermal energy is utilized to accelerate lye peeling activities [15, 16]. The dry-caustic peeling combines the effects of chemical reaction and thermal shock to soften the skin so as to reduce the use of water and chemicals during the peeling process [17, 18]. As a result, it has fewer waste disposal concerns in comparison with the conventional wet lye peeling method.

Wet lye peeling is by far the most popular technique used in commercial peeling processes, and it has been applied for a wide variety of fruits and vegetables, like tomatoes, sweet potatoes, potatoes, peaches, guava, pears, and apricots. Its great suitability for various products, the ease of process control and automation, high peeling quality, and efficiency are practical advantages for industrial-scale operation [9]. Product immersion time in the lye solution, temperature of the hot lye bath, and lye concentrations are the three major controllable factors that affect the total usage of chemicals and final peeling quality [19, 20]. Lye diffusion coupled with heat penetration may cause cellular injury and discoloration in tissues adjacent to the skin, resulting in a "heating ring" appearance, which can become quite pronounced for certain products such as potatoes, sweet potatoes, and peaches, and considerably harm the sensory quality. Effluents from lye peeling containing organic loads (BOD and COD) with high pH values (11–13) cannot be directly released to the environment without appropriate neutralization treatments [3]. The waste disposal problem and serious salinity contamination inherent to the traditional wet lye peeling process become the major issues for food processors [21].

3.2.3 Steam Peeling

The steam peeling technique involves placing raw fruits or vegetables inside a pressure vessel, and exposing them to high temperatures and pressurized steam for rapid heating in a short period (15–30 s). As the pressure is released, thermodynamic changes at the product surface cause peel detachment [12, 22]. The loosened skins are subsequently removed by pressurized water spray or mechanical pinch rollers [12]. As a chemical-free method, steam peeling has been increasingly adopted by the food industry as a replacement for the lye peeling of potatoes, tomatoes, pimiento peppers, sweet potatoes, and other vegetables [12, 23–25]. The main advantages of steam are the reduction of chemical contamination and the reduced salinity issue in wastewater treatment as compared to lye peeling. Inferior appearance, decreased firmness, and high mass losses are the major drawbacks of the commercialized steam peeling technique. Modifications of the conventional steam method, such as high-pressure steam peeling with flash cooling, lye–steam peeling, and freeze–heat peeling, were investigated in the past number of decades, targeting the minimization of peeling loss and improvement in the quality of peeled products [1, 3].

3.2.4 Flame Peeling

In this thermal-based peeling method, a flame is used to scorch the outer layer of products at extremely high temperatures

(>1000°C) in a controlled short time frame (1–3 s) [3]. Products are passed through a furnace tunnel equipped with gas burners that are designed to provide adjustable and adequate thermal intensity in a uniform manner. Flame peeling is applied mainly to fruit and vegetable materials that contain extremely high moisture content, such as peppers, garlic, and onions [6, 26, 27]. Sufficient heat capacity of materials with high moisture content allows relatively easy burn-off of the outer skin of the material without overheating the product's internal flesh [10]. The main advantages of flame peeling are (1) it can reduce the microbial populations, thus increasing the shelf stability, and (2) it can preserve the ascorbic acid content. Since flame peeling involves the relatively complicated construction, installation, and maintenance of equipment, the high capital outlay precludes its adoption by small-scale food processors [27].

3.3 EMERGING PEELING TECHNIQUES

Over the years, novel and sustainable peeling techniques have been increasingly studied and developed to reduce the usages of chemicals, energy, and water in the conventional peeling methods. Recently reported alternative peeling techniques include infrared dry-peeling, enzymatic peeling, ohmic peeling, and ultrasonic peeling.

3.3.1 Infrared Peeling

Infrared peeling has been developed as a sustainable and promising peeling technique that can eliminate the use of any chemicals and any heating media like hot water or pressurized steam to promote skin separation [2, 28]. Rapid heating of the product surface at a low heat penetration depth (<1 mm) is achieved by utilizing non-ionizing radiation from far- and mid-infrared wavelengths. As a result, only a shallow layer of product surface is subjected to the intense infrared radiation, which causes the skin to loosen enough to be washed away easily. Because of the rapid surface heating characteristics of infrared, the edible inner part of the product stays at a low temperature (<30°C) during infrared peeling, thus maintaining minimal changes in the quality and nutritional values of the peeled product [29, 30]. Since no water is used during infrared heating, this process is referred to as the infrared dry-peeling method [28]. When compared to lye peeling outcomes, infrared dry-peeling produces similar peelability, less weight loss, and comparable product firmness and appearance [31].

Bench-scale and pilot-scale infrared dry-peeling systems were designed, built, and tested for tomatoes, pears, and peaches [21, 32]. Evaluations of a gas-based flameless catalytic infrared peeler and an electricity-based infrared peeler were conducted in pilot-plants and multiple commercial tomato processing facilities over several growing seasons (2010–2018) [9]. The infrared dry-peeler consisted of three major sections: an infrared heating section, a vacuum section, and a pinch roller section [9]. Infrared-peeled tomatoes showed thinner peel-off skin, better product integrity, and firmer texture than steam-peeled tomatoes [9]. Because it is a chemical-free process, tomato skins received from infrared dry-peeling can be reutilized as a byproduct [9, 30]. Further, there is a lower cost in wastewater treatment. Given these competitive advantages, this alternative technique to lye/steam peeling could herald a step-change for the food peeling process. Enhancement of the overall heating rate and uniformity for products varying in shapes and sizes while achieving an industrially acceptable throughput need to be addressed before this novel technique becomes a commercial reality [14, 29, 33, 34].

3.3.2 Enzymatic Peeling

Enzymatic peeling is a biological peeling method. Polysaccharide hydrolytic enzymes such as pectinases, hemicellulases, and cellulases can be used to infuse into the surface of fruits and vegetables, resulting in a weakened adherence of peel to flesh because of the degradation of the pectin matrix and the breakdown of the hemicellulose–cellulose network in fruit epidermal and hypodermal layers [3, 35]. Polygalacturonase (PG) and pectin methylesterase (PME) are commonly used enzymes to hydrolyze the pectin materials in plant cell walls and middle lamella. For different fruits to be peeled, process parameters such as temperature, pH values, enzyme type, and concentrations are the key optimizable variables to achieve successful bioseparation [36]. Enzymatic peeling has been studied in several types of fruits, including citrus, grapefruit, and stone fruit [35–38]. It is advantageous that this method does not require extensively harsh treatments often seen with chemical and thermal methods [34, 36].

3.3.3 Ohmic Peeling

The ohmic heating technique was investigated for the tomato peeling process [14]. Ohmic peeling is achieved by electroheating of the tomato surface by controlling the electrical conductivity of the peeling medium, where the tomato product is immersed in a sodium chloride or sodium hydroxide solution [39]. During the ohmic peeling, each tomato acts as an electrical resistor and generates heat when electricity is passed through it. The dissipation of electrical energy into heat allows rapid and uniform heating throughout the tomato surface. Bench-scale studies showed some promise that the combined lye-ohmic peeling can improve the quality of peeled tomatoes and reduce the peeling losses and lye consumption [9].

3.3.4 Ultrasonic Peeling

Ultrasonic peeling involves using low-frequency ultrasound (20–100 kHz) to treat fruits or vegetables that are submerged in high-temperature water. The ultrasonic cavitation effect through successive compression and rarefaction of high-intensity sound waves is utilized to detach the skin from the flesh [3, 34]. The synergistic effect of power ultrasound with hot water is critical to achieving the desired peeling quality [40]. This method was primarily investigated for the tomato industry to replace the use of lye.

3.3.5 Others

Other peeling methods, such as cryogenic peeling, vacuum peeling, acid peeling, and peeling with ammonium salts or calcium chloride, have been investigated in the past few decades [1]. These methods can be considered as modifications of the above-mentioned conventional methods. Successful commercialization of these other alternatives has been hampered due to the low throughputs and/or high processing costs [3].

3.4 PEELING FUNDAMENTALS

The mode of action associated with any chemical- or thermal-based peeling process usually combines biochemical and biophysical changes occurring at the product's outermost surface and adjacent inner layers. In steam peeling, for example, thermal shock induced by the pressurized steam strikes on the product's outermost surface first, causing the melting and reorganization of cuticle waxes at the product's epidermal layer which is known as the phase transition [9, 12, 41]. As the heat transfers from the surface into the product's inner tissue, the increased temperature leads to the cell wall rupture. This vaporization of cellular fluids moves to the adjacent epidermal layer, thus accelerating various biochemical reactions at the product's inner layers. The occurrence of various biochemical reactions (e.g., hydrolysis of polysaccharides and degradation of pectin) results in microstructural changes in the product's epidermal and hypodermal layers, and finally causes the separation of skin from flesh [11, 12, 31]. After peeling, changes in product quality, such as texture and nutritional content, are attributed to the thermal softening or chemical degradation in the peeling process, where a kinetic modeling approach is typically employed to describe any chemical process within the product's surface tissues [42, 43]. In terms of transport phenomena, many peeling processes involve complex heat and/or mass transport processes inside and outside of the food product that may possess a unique skin structure. Knowledge of the skin anatomy and fruit physiology can facilitate the evaluation of the multi-physicochemical transport phenomena underlying a peeling process.

An integrated approach of experimental observations and predictive modeling analysis has certainly enriched the elucidation of skin detachment behaviors in the peeling process. Numerical simulations of the transient heat transfer inside irregularly shaped tomatoes in conjugation with biomechanical measurements in the skin membrane allow an interpretation of the peel loosening and cracking phenomena (i.e., the case of infrared peeling of tomatoes) [30]. Predictions of temperature, vapor pressure, viscoelastic moduli, and shear stress evolutions in the tomato surface during the infrared heating process were quantitatively proposed and verified [13, 29, 30]. At the cellular level, a microscopic analysis evidenced that the peel loosening was observed as the melting of extracellular cuticles, collapse of surface cellular layers, thermal expansion, and severe degradation of cell wall structures. These factors contribute to the increased peel stiffness and reduced peel adhesiveness [30, 31]. Skin crack behaviors were attributed to the rapid surface heating of infrared radiation, which reduced the skin failure strength and caused a build-up of vapor pressure under the skin membrane. When the vapor accumulated to a certain level, skin cracking can occur as the shear stress in the skin membrane exceeds the critical rupture stress [30].

Mechanistic understandings of a peeling process can provide valuable insights into the development of new peeling processes and the design of new peeling equipment. For example, in the design of the first infrared peeler where the infrared heating section must accommodate tomato populations of various shapes and sizes, curve-shaped infrared emitters were custom-made to match the fruit's geometric features. The curve-shaped infrared emitters promote intensive and uniform surface-to-surface radiation that can rapidly heat the fruit surface from room temperature to boiling temperature in about a few seconds [9, 21]. Immediately after infrared heating, a vacuum chamber was engineered to increase the formation of cracks by enlarging the pressure difference across the skin membrane, which facilitates peel cracking, allows easier subsequent peel removal and results in better peelability. Clearly, the design of peeling processes and equipment would benefit from a sound mechanistic understanding of the peeling fundamentals. Understanding the peeling basics can not only help the successful peel release but also prevents potential loss of nutritional content.

3.5 PEELING PERFORMANCE AND PRODUCT QUALITY

An adequate peeling process must be established to (1) achieve satisfactory peel removal with maximized peeling efficiency, (2) produce premium-quality peeled product, (3) minimize peeling loss, product quality changes, and pollution hazards resulting from a peeling process, (4) reduce the consumption of chemicals, water, and energy, and (5) save time, labor, and economic cost [1, 29]. In practice, reliable assessment of the peeling performance of incoming raw products is a challenge due to the substantial amount of variability in raw product physicochemical properties, cultivars, product defects, seasonal variations, and many other agronomic factors [30, 44]. Peeling evaluation can consist of both objective and subjective quantifications. A general guideline is that using a single grading scale or criterion in peeling evaluation can introduce bias and should be avoided [14, 30]. Instead, the creation of an inclusive metric from different efficiency, quality and product safety perspectives allows better and comprehensive assessments of the peeling process [31]. Some commonly used criteria include the peelability, peeling yield/loss, the ease of peeling, percentage of peel removal, peeled skin thickness, peeling residence time, peeling efficacy, peeling throughput, efficiency of water usage, energy consumption, and so on [11, 21, 31, 45, 46]. Additional consideration may also be given to overall economic efficiency. Different evaluation matrices can be developed to quantify the peeling performance, such as raw materials to be peeled, and the desired final products.

Commercial processors are not concerned with only the peeling performance but also the safety and quality of peeled products. During peeling, removal of the skin induces

mechanical injuries in fruits and vegetables due to cellular damage of the outer pericarp surface that protects the inner edible tissue. Levels of food safety and quality are crucial concerns in peeling processes. Because the increased susceptibilities to deterioration such as enzymatic changes and microbial contaminations can take place at the peeled surfaces anytime during food processing and handling, the quality and shelf-life of peeled products may be compromised [6, 47, 48]. Accordingly, industrial quality controls of peeled products include visual appearance, texture, flesh color, taste, nutrient loss, integrity of peeled product, and so on. A sampling protocol for incoming products and fruit tagging procedures, like the radio frequency identification (RFID) method, are practical options to monitor closely any quality changes throughout the peeling process [14]. Novel techniques for quality control by means of digital imaging analysis, analytical spectroscopic techniques, dynamic thermal analysis, and magnetic resonance imaging have been explored in order to achieve the best quality assurance and management [13, 49, 50].

3.6 PEELING SUSTAINABILITY

Conventional peeling operations can consume extensive amounts of water and energy, and inevitably generate waste that requires additional waste management to avoid contaminating the environment [4]. In recent years, the long-term water supply concerns and the enforcement of wastewater discharge regulations have exerted environmental pressure and financial burden on food processors [28, 34, 51, 52]. Such new challenges for the fruit and vegetable processing industry have created an incentive and strong desire to develop sustainable and cost-effective peeling alternatives that can reduce the peeling wastage, water and energy usage, and overall cost of the peeling process [9, 31]. Overall, the development and appropriate selection of peeling methods need to consider the sustainable aspects in terms of reducing energy and carbon footprints, minimizing chemical contamination, and improving water-use efficiency.

3.7 FINAL REMARKS

Peeling is a widely used process to produce various premium-quality products in the fruit and vegetable industry. As a unit process, peeling can be water- and energy-intensive. The selection of a proper peeling method for different fruits and vegetables can impact the high-level production of finished products: not limited to the product quality and safety, but also the inherent waste management and operating expense. Most popular industrialized processes that were developed a few decades ago may result in enormous amounts of peeling effluents with high costs of wastewater handling and disposal. Future endeavors would be engaged with the development of sustainable and cost-effective peeling alternatives that can reduce water, chemical, and energy consumptions and minimize wastewater generation while producing high-quality products. A holistic approach must be taken to optimize any emerging peeling technique that can be effectively and economically applied to a wide range of food products.

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4 Postharvest Physiology of Fruits and Vegetables

V. K. Mishra and T. V. Gamage

CONTENTS

4.1 POSTHARVEST QUALITY

Fruit and vegetables are consumed in fresh and processed forms (canned, frozen, dried, preserves, fermented products) and are valued for their health-promoting potential. Kader [1] defined quality as "a combination of characteristics, attributes, or properties that give the commodity value as a human food." However, the definition varies along the supply chain which consists of producers, handing and distribution agencies, processors, and finally a consumer. Quality attributes normally used for raw materials are: physical (size, firmness, presence or absence of seeds, etc.), compositional (natural sugars, volatiles), nutritional (vitamins, antioxidants, functional components), and sensory (color, texture, taste, flavor, and odor). Specific quality requirements of raw material vary with the nature of the product and processing applied to it. Reasonable storage life of fruits and vegetables before processing is an additional criterion used by processors to assess their suitability as a raw material and to increase the processing season. Minimization of deterioration of quality of plant produce intended for processing is the main aim of suppliers of fruits and vegetables that are stored and handled. Postharvest quality is an important determinant of the quality of fresh and processed fruit and vegetable [2]. As depicted in Figure 4.1, the quality of fruit and vegetables depends on several factors that can be classified as preharvest, harvesting, and postharvest. The physiological status of these live and morphologically diverse products, in addition to diseases and pests, and mechanical injuries, and injury during postharvest handling, impacts directly on quality. Knowledge of postharvest physiology is therefore fundamental to understanding the process of deterioration in order to prevent postharvest losses in terms of both quality and quantity for both fresh and processed product markets.

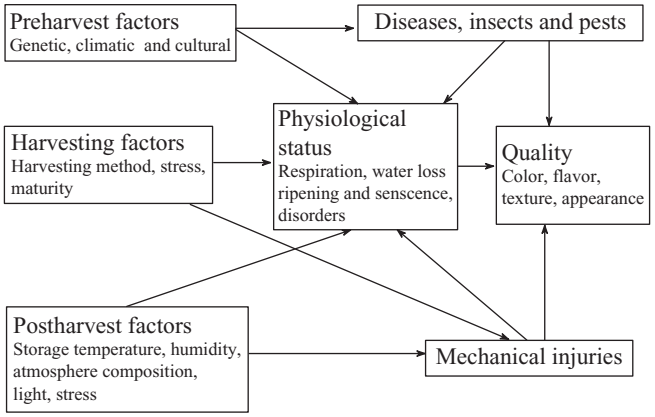

FIGURE 4.1 Schematic representation of factors affecting postharvest quality of fruits and vegetables.

Postharvest period begins at the separation of plant organ used as food from the medium of its immediate growth or production and ends when it enters the process of preparation for final consumption or further preservation and processing. Fruit and vegetables are live tissues harvested at various stages of their growth and development, have tender texture, contain high moisture content (60–95%) and water activity, lose water to the surrounding atmosphere, and continue respiration, which produces heat and water at the expense of food reserves, which were otherwise replaced by photosynthates and nutrients supplied by the plant before harvest. In this chapter, we provide an overview of postharvest physiology processes in relation to the postharvest quality of fruits and vegetables.

4.2 FACTORS AFFECTING QUALITY

4.2.1 Preharvest Factors

Preharvest factors determine most of the quality of plant produce including fruits and vegetables, and postharvest management must ensure that harvest quality deterioration is minimized. In fact, 70% of the quality of fruits and vegetables depends on preharvest conditions [3–5].

4.2.1.1 Genetic

Genetic makeup has a profound effect on the selection of a raw material for a given processing application. Cultivar and rootstock selection influence the composition, quality, storage potential, and response to processing. Fruit cultivars grown for fresh market sale may not be suited for processing and vice versa. The criteria used by breeders in the development of new varieties are high yield, resistance to disease and disorders, improved compositional and nutritional values, reduction in undesired toxic compounds, and improved processing characteristics. Selection of cultivar is important for both enhanced storage life and optimal quality of the fruit or vegetable for processing. Varieties have been identified to suit a particular processing method [6–11]. For example, the Roma variety is more suitable for canning than juicy and acidic varieties of tomato. Fruit and vegetable processors usually contract out growers who grow a particular variety that suits the raw material specifications for a given type of processing.

TABLE 4.1
Transgenic Fruits and Vegetables Released with Improved Quality Claims

Produce	Trait
Apple	Reduction in the incidence of bitter pit, reduction in browning (Arctic apple)
Banana	Delayed ripening, increased bruise resistance
Melon	Altered ripening
Eggplant	Seedless
Cucumber	Seedless
Pepper	Altered ripening, improved flavor
Potato	Reduced bruise sensitivity, increased amylopectin
Strawberries	Delayed softening and ripening
Tomato	Increased solid content, delayed ripening, increased shelf life

There has been a significant amount of work reported on modification of genetic make-up to improve the postharvest performance of fruits and vegetables [7–9]. Table 4.1 lists the transgenic fruits and vegetables released and traits targeted to improve postharvest quality to reduce browning and softening tendencies, increase storage life [7] to enhance processing time, and improve uniformity of the flavor and color to ensure consistency of processed product quality.

4.2.1.2 Climatic

The climatic conditions, such as temperature, humidity, light, wind, soil texture, elevation, and rain fall, significantly influence fruit and vegetable quality [5, 12, 13]. The duration, intensity, and quality of light during cultivation affect the quality at harvest. In tomatoes, leaf shading of fruits results in a deeper red color during the ripening and when grown in full sunlight they contain more sugar and dry matter. Exposure to sun tends to make citrus fruits lighter in weight, with thinner rind, low amounts of juice and acids, and high solid content compared to those that are shaded or those inside a canopy. In purple cabbage and eggplants, formation of anthocyanin pigments is controlled by short wavelengths of light in the blue and violet regions [14]. Thiamine synthesis is stimulated by light and generally occurs in the leaves and increases in concentration until the plant is mature. Turnips harvested in the morning contain more riboflavin than those harvested at other times of the day [13]. Fruits grown in cold climates usually are more acidic than those grown in warmer regions [15]. Moretti et al. [16] reviewed literature on the effect of climate change (increase in temperature, CO_2, ozone) on quality of fruit and vegetables. Temperature can influence photosynthesis, respiration, aqueous relations, membrane stability, plant hormones, and primary and secondary metabolite synthesis through enzyme-mediated biochemical reactions. For plants to have a higher yield, the photosynthetic rate needs to be high and the ratio of photosynthesis/respiration rate should be higher than one. An increase in temperatures will reduce

maturity time [16]. High CO_2 levels result in the malformation of potato tubers, increased incidence of scab, and a change in reducing sugar content. Increase in ozone reduces photosynthesis and alters synthesis of other metabolites. For example, exposure to 0.005 to 1.0 mol/mol ozone results in a transient increase in the β-carotene, lutein, and lycopene contents of tomatoes; a high level of ozone increases vitamin C content and decreases emissions of volatile esters in strawberries [16].

4.2.1.3 Cultural Practices

Soil nutrient and water supply, pruning, thinning, pest control or chemical spray, and density of planting influence the quality of plant produce [12, 17]. Fertilizer addition affects the mineral content of fruit, while other cultural practices such as pruning and thinning may influence nutritional composition by changing fruit crop load and size [18]. The closer the planting, the less sweet will be the fruits. Potatoes grown in sandy, gravelly, or light loamy soils, and low-water or -fertility soils have higher dry matter. A high N/K ratio and phosphorus deficiency in soil increase the tendency of potato to darken after cooking. Pineapple plants receiving undue amounts of nitrogen produce tart, white, and opaque fruits with poor flavor characteristics [18]. Pesticide residues may give rise to flavor taints in fresh and processed products and may even produce harmful metabolites and toxicity. Plant nutrient type and the amount of fertilizer applied have an impact on tomato color, sugar levels, total soluble solids, flavor, and texture and are related to susceptibility to diseases [18].

4.3 HARVESTING FACTORS

4.3.1 Maturity at Harvest

Maturity at harvest is the most important quality criterion for a processor as it directly affects composition, quality, losses, and the storage potential of plant produce. The optimum harvest maturity is vital to achieve maximum postharvest life of the fresh produce [19, 20]. Although most fruits reach peak eating quality when harvested fully ripe, they are usually picked mature, but not ripe, to decrease injury during postharvest handling. Fruits picked either too early or too late in the season are more susceptible to physiological disorders and have a shorter storage life than those picked at mid-season [12]. Harvesting fruits when either immature or overripe can cause extensive loss of the produce; thus maturity indices are used for estimating the correct harvesting stage. The optimum maturity of produce for fresh consumption and processing is determined by the purpose for which it will be used. The maturity stage considered best for canning may not be the best for dehydration, freezing, or making jams or preserves. For example, fully ripened tomatoes should be used for the preparation of dried and concentrated products to achieve the best flavor.

Several indices are used to identify and evaluate the maturity for harvest and for assessing suitability for a processing application. Maturity indices vary among types, cultivars of the produce, and intended processing. The indices are based on: (i) change in visual appearance (size and shape, overall color, skin color, flesh color, presence of dried outer mature leaves, drying of the plant body, development of the abscission layer, surface morphology and structure, and fullness of fruit), (ii) days elapsed from full bloom to harvest, and accumulated heat units during development, (iii) physical changes (ease of separation or abscission, flesh firmness, tenderness, specific gravity or density), (iv) chemical changes (soluble solids, starch, acidity, sugar/acid ratio, juice content, oil content, tannin content), and (v) measurable physiological changes (respiration and internal ethylene concentration). Both objective and subjective methods are used in the measurement of indices. Tables 4.2 and 4.3 show commonly used indices for assessing maturity for harvesting and for assessing suitability for processing for selected fruits and vegetables, respectively. Since each method has its own limitations and advantages the accurate assessment of maturity requires the use of a combination of indices [12, 21].

4.3.2 Harvesting Methods

Harvesting can be done manually or mechanically. The harvesting system used and its management have a direct effect on the incidence and severity of mechanical injuries. Thus, management procedures should include the following for best results: (i) selection of optimum time to harvest regarding fruit maturity and climatic conditions, (ii) training and supervision of workers, and (iii) an effective quality control procedure. Pickers can be trained in methods of identifying produce that is ready for harvest [21].

Harvested vegetables other than root crops should not be placed directly on the soil and exposed to sunlight, heat, and rain. Exposure to sun can lead to a high internal temperature, which is detrimental to the quality. A simple shade or grass coverage can provide protection to the harvested products. Some root crops can benefit from brief exposure to the sun in order to dry off the surface or facilitate removal of adhering soil [18]. The time of day or night and weather conditions during picking also affect the quality. Harvesting during or immediately after rains should be avoided, and harvesting should preferably be carried out during the cooler part of the day (usually early morning) to avoid shriveling and wilting.

4.4 POSTHARVEST FACTORS

4.4.1 Humidity

Fresh fruits and vegetables contain sizable amounts of water, for example, watermelons may contain >95% water of its fresh weight. Since most of the water is free water, the produce will continue to lose water to the surrounding atmosphere. The loss in water manifests as shriveling, wilting, and loss of crispness. The tissue may also become tough or mushy and unacceptable to the consumer. The reduction of saleable weight and loss of sensory characteristics lower the marketing value. The surface area/volume ratio, nature of the surface: presence/absence of cuticle, number of stomata (leaves) and

TABLE 4.2
Harvest Maturity Indices for Vegetables and Specifications for Processing

Vegetable	Harvest	Specifications for Processing
Broccoli	Compact bud cluster	
Cabbage	Compact head	Sauerkraut (mild flavored, sweet, solid white head, Sugar >2%, ascorbic acid 30–60 mg/100 g)
Carrot	Desired length and texture	Canning (tender texture) Juicing (juice yield and sugars >6°B)
Cauliflower	Compact curd	
Cucumber	Size and tenderness	Pickles (ripe with sugar content of 1.5–2.5%)
Eggplant	Desirable size and tenderness	
Lettuce	Desirable size before flowering	
Okra	Desirable size and tips snap off easily	Canning (small, young, and tender)
Olives	Straw yellow to cheery red	Pickling (slightly less mature, size, color)
Onion	Tops beginning to dry	Drying (high solids content)
Peas	Well-filled pods which snap easily	Canning (13.4% AIS, tenderometer reading of 115–125) Freezing (13.4% AIS, tenderometer reading of 95–105) Drying (9–11% AIS, tenderometer reading of 85–95)
Potato	Tops beginning to dry	Chips (dry matter of 21–24%, specific gravity (>1.075), <1.5% sugar) French fries (specific gravity of 1.08–1.12, <0.3% reducing sugar) Frozen chips (total solids 20–22%, <0.2%) Canning (whole tubers of 19–38 mm size, specific gravity of <1.08) Dehydrated diced potatoes (specific gravity of 1.1) Starch manufacture (minimum of 15% starch)
Sweetcorn	Milky sap oozing upon pressing	Canning (slightly immature kernels)
Tomato	Seeds slip upon cutting the fruit	Most processed products (SS >5%)

Sources: Dauthy [22], Holdsworth [11], Kader [19], Kitinoja and Kader [21], Lisinska and W. Leszczynski [23], Maestrelli [24], Ranganna [25], Rodriguez et al. [26].

lenticels (fruits), periderm (tubers and roots); and injury to the plant tissues affect the rate and extent of water loss.

Water loss can be prevented by maintenance of high atmospheric relative humidity (RH), low temperature, reduced air movement, and increased pressure, avoiding product injury, and the use of suitable packaging during storage and transportation. Optimum RH is 85 to 90% for most fruits and 90–98% for most vegetables except dry onions and pumpkins (70–75%). Some roots may require almost 100% relative humidity [27, 28]. Maintaining high RH, in certain situations, may induce decay, surface mold development, and physiological disorders including impaired fruit ripening; however,

TABLE 4.3
Maturity Indices and Specifications for Processing Fruits

Fruit	Harvest	Specifications for Processing
Apple	140–150 days from the bloom, starch content	Sauce and canned products (maximum shear press values of 3.1–3.3 kN for slices, min SS of 10% for sauce)
Apricot	Three-quarters of the area of the fruit should be yellowish green or one-half yellow	Canning (full flavor) Drying (full flavor and ripening)
Grapes	14–17.5% SS, or SS/A of 20 or higher	White wine fermentation (pH 3.1–3.3, TA 0.7–0.9, Brix 19–22°B) Red wine fermentation (pH 3.3–3.6, TA 0.6–0.8, Brix 21–23.5°B)
Orange	SS/A of 8	Minimum juice content of 30–35% Frozen juice concentrate (12.5–19.5 SS/A)
Pear	>13% SS and yellowish green color	Canning (full flavor and firmness measured to 66.7–75.6 N)
Banana	Pulp to peel ratio of 1.35–1.4, or disappearances of angularity, color	Banana puree (complete disappearance of angularity, full flavor, and flesh appears translucent)
Mango	Change of peel color from green to yellow	Canning (full flavor and total sugar/soluble solids ratio close to 1)
Strawberries	>2/3 of fruit surface has pink or red color	Freezing (firmness equivalent to 10–15 N force)

Sources: Bedford [6]; Kader [28]; Maestrelli [24]; Margalit [29]; Matthews [30] Springett [31].

surface condensation of moisture (sweating) over long periods is probably more of significance in enhancing decay than high humidity.

4.4.2 Temperature

Proper temperature management is the most important tool in postharvest handling of plant produce to control both physiological and pathological deteriorations. Provided exposures to temperatures leading to chilling and freezing injuries are avoided, lowering the temperature during handling, transportation, and storage is the most effective means of extending the shelf life and reducing the loss of the quality by lowering the metabolic processes such as respiration and transpiration. Figure 4.2 shows the effect of temperature on the shelf life of selected fruits and vegetables. The difference in the effect of temperature on the shelf life varies due to differences in the physicochemical properties of different types of fruits and vegetables. For example, the most pronounced effect in increasing the shelf life by reducing the temperature is expected for lettuce and green onion rather than for strawberry and raspberry. The effect of temperature on quality is expressed by a temperature quotient, Q_{10}, which is defined as:

$$Q_{10} = \left(\frac{q_2}{q_1}\right)^{\left(\frac{10}{(T_2 - T_1)}\right)} \tag{4.1}$$

where q_2 and q_1 are the rates of quality function at two temperatures, T_2 and T_1, respectively. The Q_{10} values describe the effect of temperature on a particular quality attribute, such as color, texture, flavor, etc. For example, Q_{10} values for quality deterioration in asparagus when expressed as appearance, sugar loss, and fiber increase are 2.7, 5.8, and 10, respectively, between a temperature range 0–10°C [32].

4.4.3 Atmospheric Gas Composition

Atmospheric gas composition, such as oxygen, carbon dioxide, and ethylene, influences the microbial decay and physiological processes such as respiration. The reduction of oxygen and elevation of carbon dioxide through modified or controlled atmosphere storage complement the effects of maintaining low temperatures through the postharvest value chain. For example, transporting banana under 3% O_2 and 5% CO_2 reduces premature ripening and crown rot development by the high carbon dioxide/oxygen ratio [34]. The beneficial or harmful effects of varying gas composition, however, depend upon commodity, cultivar, physiological age, oxygen and carbon dioxide levels used, temperature, and duration of storage [28, 35]. The principles underlying the technology of manipulation of atmosphere and recommended levels of gases for extending the postharvest life of fruits and vegetables have been covered elsewhere [35–39], and in the chapter on controlled atmosphere storage (Chapter 22).

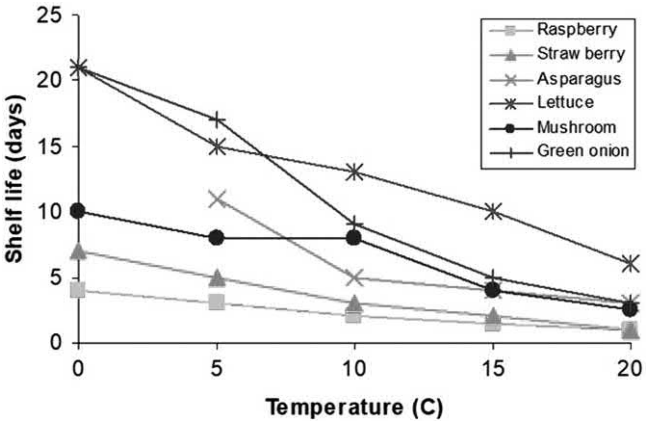

FIGURE 4.2 Effect of temperature on shelf life of selected fruit and vegetables. (Data from Brechet and Brechet [33].)

4.4.4 Light

Light may influence the quality of fruit and vegetables by controlling the synthesis/degradation of pigments responsible for color (chlorophyll, carotenoids), flavor by catalyzing the oxidation of lipids, sprouting, reducing nutritive value by degrading vitamins such as ascorbic acid and riboflavin, and production of toxins. The exposure of potatoes to light during storage may produce green tissues, which contain solanine, a toxin [18]. Thus, light intensity should be minimized. Adverse effects of light can be prevented by storage in the dark and using packaging materials that prevent the transmission of light.

4.4.5 Mechanical Injury

Mechanical injuries expose internal tissue to contamination, increase respiration rate, promote chemical and enzymatic reactions, allow the spread of decay microorganisms, and induce an overall quality decline. The surface cracks, cuts, and punctures that develop during growth or as a result of mechanical injuries weaken the protective outer layers causing water loss. At early stages of maturity, some commodities have the ability to repair and seal off the damaged area. The capacity of wound healing diminishes in most cases as the plant organs mature except some tuber and root crops.

4.4.6 Postharvest Diseases or Infections

The postharvest diseases are initiated at the early stage of development when attached to the plant, by fungi or bacterial invasion through the cuticle or through wounds, and/or natural openings in the surface, and through injuries in cut stems or damage to the surface. While most microorganisms can invade only the damaged produce, a few are able to penetrate the skin of healthy tissue. Postharvest diseases control is based on: prevention of infection, eradication of incipient infections, and retarding the progress of pathogen spread by fungicide or bactericides [40, 41].

The pH of tissue is the major factor that influences the microflora present. Yeasts and molds are often the

predominant microorganisms in fruits and fruit products as they grow well under acidic conditions. Only a few species of yeast pathogenic to man and other animals are common contaminants of fruits and fruit products [40, 41]. Mold-infected raw fruit may become soft during or after processing because pectinases present in molds despite the use of ordinary thermal treatment. For example, patulin is a common mycotoxin in apples [41].

In general, the bacteria that cause diseases in humans are not associated with fruit products due to poor tolerance to low pH of fruits. There are four groups of human pathogens that may be present in fresh fruits and vegetables [42]: soil-inhabiting bacteria (*Clostridium botulinum*, *Listeria monocytogenes*), enteric bacteria (*Salmonella* spp., *Shigella* spp, *E. coli* O157:H7, etc.), parasites (Cryptosporidium, Cyclospora), and viruses (Hepatitis, Norwalk virus, etc.).

4.5 POSTHARVEST PHYSIOLOGICAL PROCESSES

The origin and development of plants influence physiological aspects of fruit and vegetables. From initiation or conception to death (senescence), plants or parts thereof undergo different stages of development having significant differences in their metabolism, impacting on quality. Fruits and vegetables pass through five distinct developmental phases from initiation to: (i) development (morphological and chemical completion of tissue), (ii) young or premature (developmental period before the onset of maturation), (iii) mature (completion or fullness of growth and edible quality; most of the maturation processes must be completed while the produce is still attached to the plant), (iv) ripe (maximum esthetic and edible quality), and (v) senescence (leading to death and making the produce worthless and inedible). The duration and rate of these stages vary with the type and variety of the product and stage of development [1–3 13, 19–21, 28, 35]. Table 4.4 provides examples of plant parts used as fresh or in processed forms. The morphology and the stage of development at which these are harvested vary substantially. When harvested, sprouts and seedlings are in the very early stages of development, followed by stems and leaves, partially developed fruits (cucumber), fully developed fruits (apples), roots and tubers and seeds (dry beans, peas). Tissues that are in the early stage of development have relatively short shelf life as compared to those which are primed for dormancy (e.g. potatoes).

TABLE 4.4
Types of Plant Parts Consumed in Fresh or Processed Form

Plant Part	Example
Seeds and pods	Peas, chick pea, corn, baby corn, beans, okra
Bulbs, roots, tubers, and corms	Potato, beet, carrot, onions, garlic, lotus root, cassava
Flowers	Cauliflower, broccoli, banana flower
Fruits	Tomato, pears, peach, pineapple, olive, eggplant, mango, lychee
Buds	Bamboo shoot
Stems	Asparagus
Leaves	Lettuce, spinach
Seedlings	Bean, alfalfa, onion, radish sprouts

The unwanted sprouting in vegetables, such as onions, ginger, garlic, and potatoes, is related to dormancy and rest. Dormancy is a condition of quiescence due to some internal or external factors, and rest is a phenomenon in which sprouting does not occur in spite of a favorable environment (for example, potatoes have no period of rest) [18]. Rooting in roots and tubers is initiated by high humidity in the environment and may result in rapid decay, shriveling, and depletion of food reserves [13, 35]. Seed germination of mature fruits during storage, such as chayote, tomatoes, papaya, and pod-bearing vegetables, is also a serious problem. Green beans and sweetcorn may toughen due to the development of spongy tissues when storage is unduly prolonged [18]. Elongation of existing structure as in asparagus, carrot, beet, and kohlrabi, and bending of tissues in response to gravity and light reduce the market life.

4.5.1 Respiration

All living organisms convert matter into energy through a fundamental process of life called respiration, which primarily constitutes enzymatic oxidation of substrates, such as carbohydrates, proteins, lipids, organic acids, etc., in the presence of atmospheric oxygen to carbon dioxide and water, and accompanied by a release of energy as follows:

$$C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O + 2872\,kJ \qquad (4.2)$$

The reaction brings about changes in the chemical composition as carbohydrates, proteins, lipids, and organic acids are used as substrates. The respiratory quotient (RQ), which is defined as a ratio of CO_2 produced to O_2 consumed, can indicate the type of substrate being oxidized during respiration. The RQ values for carbohydrates, organic acids, and lipids are 1, >1, and <1, respectively. The prevalence of anaerobic respiration corresponds to high RQ values. The respiration reaction is exothermic as a significant part (about 57%) of the energy produced is dissipated as heat, called vital heat or heat of respiration, which contributes to a further increase in the temperature of the commodity.

Respiration plays a significant role in the postharvest physiology and deterioration of quality of plant foods. The rate of deterioration is generally proportional to their respiration rate, which often is a good index of the storage potential of a fruit or vegetable. The higher the respiration rate the shorter is the shelf life and vice versa. The respiration rate can be used as a criterion to compare the perishability of fruits and vegetables. Kader and Barrett [12] classified fruit and vegetables into five groups based on their respiration rate as shown in Table 4.5, which also illustrates the relationship between the relative perishability and their respiration rates. Commodities such as mushrooms, which respire at rates three times those of dried fruits, are more perishable and have a shorter shelf

TABLE 4.5
Classification of Fruit and Vegetables Based on Respiration Rate

Class	Respiration Rate (mg/kg hr) 10°C	20°C	Examples
Very low	<10	<40	Nuts, dates, dried fruits
Low	10	40	Potatoes, onions, cucumbers, apple, pear, kiwi fruit, pomegranate, Chinese date
Moderate	10–20	40–80	Peppers, carrots, tomatoes, eggplant, citrus fruits, banana
High	20–40	80–120	Peas, radish, apricot, fig, ripe avocado, cherimoya, papaya
Very high	>40	>120	Mushrooms, green onions, cauliflower, dill, parsley, melons, okra, strawberry, blackberry, raspberry

Source: Kader [12].

life than nuts and dried fruits. The effect of respiration on perishability can be explained by (i) coupling of the resultant reducing power and formation of ATP due to respiration with biosynthetic reactions leading to loss of quality, (ii) loss of food reserves, (iii) toxic effects of accumulation of carbon dioxide, and (iv) increase in product temperature due to respiratory heat, which dictates the cooling load requirement during storage [39, 44, 45].

Useful data on experimental respiration rates for different fruits and vegetables are given by several references [45, 47–49]. Experimental data on the production of CO_2 are correlated to temperature by a relationship such as:

$$\dot{m}_{CO_2} = f \times \left[\frac{9T_m}{5} + 32\right]^g \tag{4.3}$$

where $\dot{m}_{CO_2}$ is the rate of carbon dioxide production per unit mass of the product, T_m is the mass average temperature of the product (°C), and f and g are the respiration coefficients for a given product. The coefficients obtained by least square fit of the experimental data are given in Table 4.6 [47]. Similarly, the relationship between the heat of respiration and temperature can be derived from the reaction stoichiometry involving glucose oxidation. Within the physiological range of temperatures (0–30 °C), the rate of respiration increases exponentially (Figure 4.3) and a large amount of heat is produced as heat of respiration (Figure 4.4).

The respiration rate depends on a host of internal and external factors. The internal factors include (i) the quantity of substrate (predominantly sugars), (ii) the size, shape, cell morphology, and maturity, (iii) the structure of the peel, (iv) the volume of intercellular spaces, and (v) the chemical composition of tissue which affects solubility of oxygen and carbon dioxide. The external factors are: (i) temperature, (ii) availability of ethylene, oxygen, carbon dioxide, (iii) light, (iv) water stress, (v) biological activity, and (vi) growth regulators.

TABLE 4.6
Respiration Coefficients (f and g) for Equation 4.3 for Selected Fruits and Vegetables

Fruit or Vegetable	f	g
Apples	5.687×10^{-4}	2.598
Blueberries	0.002724	2.573
Cabbage	6.080×10^{-4}	2.618
Carrots	0.05002	1.793
Grapes	7.056×10^{-5}	3.033
Limes	2.983×10^{-8}	4.733
Onion	3.668×10^{-4}	2.538
Oranges	2.805×10^{-4}	2.684
Peaches	6.361×10^{-5}	3.204
Pears	6.361×10^{-5}	3.204
Potatoes	0.01709	1.769
Strawberries	3.668×10^{-4}	3.033
Tomatoes	2.007×10^{-4}	2.835

Source: Becker and Fricke [47].

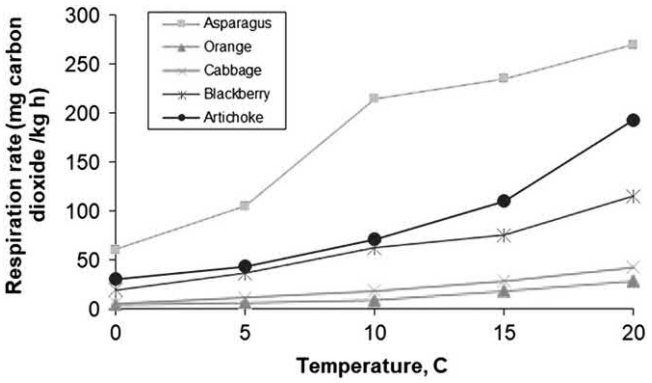

FIGURE 4.3 Effect of temperature on the respiration rates of some fruit and vegetables. (Data from Gross et al. [45].)

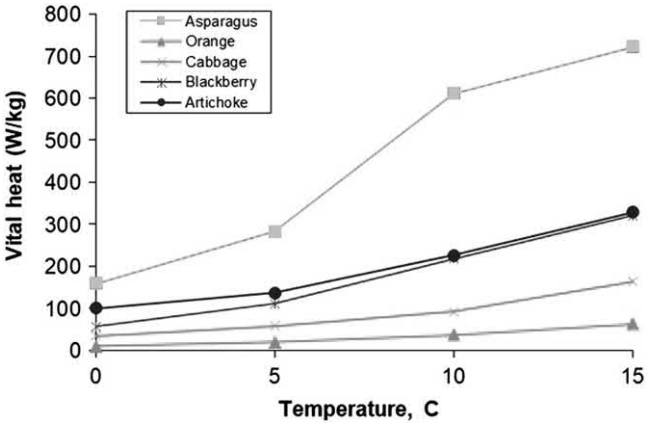

FIGURE 4.4 Effect of temperature on the evolution of vital heat by some fruit and vegetables. (Data from ASHRAE [44].)

Out of these external factors, temperature, atmospheric composition, and physical stress have the most profound effect on respiratory activity, and postharvest management of respiration involves controlling these factors to reduce the deterioration of quality.

As the produce approaches maturity, the rate of respiration declines, and those commodities that are harvested while in the period of active growth (e.g. most vegetables and immature fruits) have high respiration rates. However, fruits show two distinctive respiratory patterns during ripening and are grouped into: (i) non-climacteric, and (ii) climacteric. Climacteric fruits show a dramatic increase in the rate of respiration during ripening. The climacteric peak can be prolonged or delayed by reducing the rate of respiration in order to increase the shelf life. Climacteric fruits can be harvested mature and ripened off the plant. These produce much larger quantities of ethylene in association with their ripening, and exposure to ethylene treatment will result in faster and more uniform ripening [12]. The respiration rate is at a minimum at maturity, and remains rather constant, even after harvest. The rate will rise up abruptly to the climacteric peak only when ripening is about to take place, and then it will slowly decline. The non-climacteric fruits are not capable of continuing their ripening process once removed from the plant. These produce very small quantities of ethylene, and do not respond to ethylene treatment for ripening, except in terms of degreening (degradation of chlorophyll) in citrus fruits and pineapples [12]. Examples of non-climacteric and climacteric fruits and vegetable are given in Table 4.7.

The respiration rate depends on temperature, and the temperature dependence varies among and within commodities. The relationship between temperature and respiration rate can be expressed in the form of Q_{10} values, which allow the estimation of the rate of respiration at an unknown temperature provided the rate of respiration at a known temperature is available. The temperature quotient, Q_{10}, for respiration may be defined by replacing q_2 and q_1 by R_2 and R_1, respectively, in Equation 4.1. R_2 and R_1 are the rates of respiration at two temperatures, T_2 and T_1, respectively. Q_{10} values for respiration rates are not constant and vary between 2 and 2.5 for a temperature range of 5–25°C [32, 35, 48]. As the temperature increases, Q_{10} values decrease, and Q_{10} tends to reduce to less than 1 as the thermal death point of the tissue approaches [48].

Reduction in the O_2 concentration and increasing concentration of CO_2 in the atmosphere surrounding fresh fruits and vegetables reduce the rate of respiration. The extent of the effect depends on factors such as temperature, produce, cultivar, age, and level of maturity at harvest. A 3–5% reduction of oxygen concentration does not have an adverse effect on produce, but a comparable increase in carbon dioxide may suffocate and ruin certain fruits and vegetables. There is considerable variation in the injury threshold of various fruits and vegetables to O_2. Reduction of O_2 concentration below 2–3 % gives a beneficial reduction in rates of respiration and other metabolic processes for most produce. However, complete removal of O_2 is not recommended as an anaerobic environment is detrimental to the quality of the produce as it leads to fermentation, decay and development of off flavor, and change in color and texture.

The stage of development of produce also influences the rate of respiration. The rate of respiration declines with maturity; immature fruits respire at a higher rate than those that have attained full maturity. Actively growing vegetables such as asparagus (floral meristem) have very high respiratory rates compared to storage organs such as potatoes and mature fruits (Figure 4.3). This is the reason why potatoes can be stored for longer periods than asparagus.

Physical stress during cultivation, harvesting, and postharvest handling influences respiratory behavior significantly. Tissue injury increases the rate of respiration and induces ethylene production, which may further catalyze an increase in respiration with consequent loss of quality. While assessing the relationship between respiration, bruising susceptibility, and temperature in sweet cherries, Crisosto et al. [17] found that impact bruising damage was greatest in the tested cultivars when fruit flesh temperature was below 10°C.

TABLE 4.7
Examples of Non-Climacteric and Climacteric Fruit and Vegetables

Non-Climacteric	Climacteric
Berries (cherry, strawberry, blueberry, cranberry, raspberry), citrus fruits (orange, grapefruit, lemon, lime, mandarin), pineapple, lychee, tamarillo, loquat, cucumber, fig, melon pomegranate	Apple, pear, quince, persimmon, apricots, nectarine, peach, plum, kiwi, avocado, banana, plantain, mango, papaya, cherimoya, sapodilla, guava, passion fruit, pawpaw, tomatoes

Sources: Kader [12].

4.5.2 Transpiration and Water Stress

Transpiration is mainly responsible for water loss, which leads to a loss of: salable weight, appearance (wilting and shriveling), textural quality (softening, flaccidity, limpness, crispness, and juiciness), and nutritional quality [12]. In most fruits and vegetables, a 5 to 10% loss in moisture content produces visible symptoms of shriveling and wilting due to cellular plasmolysis [18, 35].

Transpiration is a process of mass transfer in which the water vapors move from the surface of the produce to the surrounding atmosphere. The rate is directly proportional to the partial pressure gradient across the transfer surface area and inversely proportional to the sum of the resistances, such as the type of the surface and presence of waxes on the surface. The simplest mathematic model of the transpiration process used in the literature is of the following type,

$$\dot{M} = k_t(p_s - p_a) \tag{4.4}$$

where $\dot{M}$ is the transpiration rate, k_t the transpiration coefficient, and p_a and p_s are the ambient and evaporating surface vapor pressures, respectively. The transpiration coefficient represents the reciprocal of resistances to moisture transfer. The experimental data on transpiration coefficients have been compiled by Sastry et al. [51] and are given in Table 4.8. Such coefficients have limitations in describing the true transpiration process. Further improvements of the transpiration model were suggested by Sastry and Buffington [50] and Becker and Fricke [47]. It may be possible to group fruits and vegetables into three broad ranges of transpiration rates under refrigerated storage conditions: high (500–850 mg/kg hr mm Hg, carrots and parsnips), intermediate (100–250, cabbage and rutabagas), and low (10–80, potatoes and onions). The rate of transpiration depends on both product and environmental factors. These factors include (i) skin structure, (ii) size, shape, and surface area, (iii) water vapor pressure difference, (iv) air movement, (v) heat of respiration, (vi) the level of maturity, (vi) endothermic effects of evaporation, and (vii) the amount of solutes present in the produce.

The main sites of transpiration in plants are the hydathodes, stomata, epidermal cells, lenticels, trichomes (hairs), and cuticle. The number of stomata in the epidermis, type of surface, tissues under the skin, and structure and thickness of wax coating on the surface (cuticle) determine loss of water. The higher the ratio of surface area to volume, the greater is the loss of water by evaporation. Thus, at the same conditions the expected rate of transpiration will be in the order leaf (spinach) > fruit (tomato) > a root or a tuber (potato). Immature fruits tend to encounter higher transpiration rates than mature fruits due to the higher permeability of the skin to water vapors. Since the solute depresses the water activity of solutions, higher solute concentration in the tissue binds water and reduces water loss.

TABLE 4.8
Transpiration Coefficients of Selected Fruit and Vegetables

Product	Mean Transpiration Coefficient (mg/ kg s mPa)	Range of Transpiration Coefficient (mg/ kg s mPa) Reported in the Literature
Apples	42	16–100
Brussels sprouts	6150	3250–9770
Cabbage	223	40–667
Carrots	1207	106–3250
Celery	1760	104–3313
Grapefruit	81	29–167
Grapes	123	21–254
Leeks	790	530–1042
Lemons	186	139–229
Lettuce	7400	680–8750
Onions	60	13–123
Orange	117	25–227
Parsnips	1930	1097–2771
Peaches	572	142–2089
Pears	69	10–144
Plums	136	110–221
Potatoes	25	15–40
Tomatoes	140	71–365

Source: Sastry et al. [51].

The temperature, relative humidity, and air movement are the three most significant environmental factors that affect water loss by transpiration. In general, high surface temperature and low relative humidity increase the rate of transpiration. As is indicated by Equation 4.4, the vapor pressure difference, the driving force for water movement, has a direct relationship with the transpiration rate, and therefore the difference can be used to determine the transpiration rate. Sastry and Buffington [50] reported the following regression equation (Equation 4.5) for the best fit ($R^2 = 0.99$) curve describing the transpiration rate and apparent vapor pressure difference for tomatoes:

$$\dot{m} = 0.038557\,(p_{sa} - p_{\infty})^{0.9536} \tag{4.5}$$

where $\dot{m}$ = the rate of transpiration (mg/cm^2 s), p_{sa} = apparent water vapor pressure at the evaporating surface, and p_{∞} = atmospheric vapor pressure in mm Hg. The relationship for tomatoes has been almost linear. While atmospheric vapor pressure is dependent on both temperature and air relative humidity, the apparent vapor pressure depends mainly on temperature due to saturation conditions prevailing in the tissue. Thus, one practical way to minimize transpiration is to cool the produce quickly under high humidity conditions (hydro-cooling). Respiratory activity produces heat that contributes to higher evaporation of water even under prevailing saturation conditions by increasing the vapor pressure deficit. It is expected that fruits and vegetables, which have higher respiratory activity, will also have higher water loss. The effect of maturation and ripening on transpiration varies significantly amongst fruits. Fruits such as mango and banana show increased transpiration after reaching the climacteric period [14]. The dissolved solutes, particularly simple sugars at higher concentrations, reduce the vapor pressure of water in the solution inside the tissues more and hence reduce the rate of transpiration. Air circulation or velocity increases the moisture evaporation from the surface, particularly in situations where temperature fluctuations persist.

Water loss can be minimized by maintaining higher pressure than atmosphere, maintaining low temperature and humidity during storage, loading density and depth, application of waxes and other water-resistant coatings to the surface, or by appropriate packaging such as with plastic films [35, 47, 52].

4.5.3 Ripening and Senescence

Ripening refers to a stage in tissue development when a fruit reaches an optimal eating quality, as evidenced by favorable change in composition, color, texture, and other sensory

attributes [19, 35]. Many fruits (climacteric) require ripening to be carried out by the processor as fruits have been shipped to processors in the immature stage to avoid tissue injury during transportation and handling. Ripening in fruits follows physiological maturity and precedes senescence, which leads to the death of the tissue. Senescence is genetically programmed and can be induced by common stressors such as tissue injury, deficiency of nutrients and water during production, exposure to insects, pests, and diseases, and adverse environmental conditions. An understanding of the biochemistry of senescence, therefore, provides clues to delay the loss of postharvest quality of fruits and vegetables. King and O'Donoghue [53] identified three main areas of research conducted to unravel senescence, the role of ethylene, structural changes in the cell wall, and metabolic changes when immature after harvest.

Ripening induces changes that are structural, physical, chemical, nutritional, bio-chemical, or enzymatic. These changes are (i) degradative, such as chlorophyll breakdown, starch hydrolysis, and cell wall degradation, and (ii) synthetic, such as formation of carotenoids and anthocyanins, aroma volatiles, and ethylene formation.

The details of compositional and morphological changes during ripening and senescence are given in several references [1, 12, 13, 19, 33, 35, 46, 54]. Carbohydrates, organic acids, amino acids and proteins, lipids, pigments, pectic substances, and volatile components are mostly affected and directly contribute to the pleasant color, aroma and flavor, texture, and appearance of fruits. The flesh cells enlarge and sugar content increases at the expense of starch, acid, and phenolic compounds as the fruits approach ripening. In addition, certain volatile compounds develop, giving the fruit its characteristic aroma and flavor [54]. Chlorophyll degradation (loss of green color) and synthesis of carotenoids (yellow and orange colors) and anthocyanins (red and blue colors) take place both in the skin and in the flesh. All fruits soften as they ripen due to changes in cell wall composition and structure. The pulp weight increases with the gradual decrease in peel weight upon ripening. Figure 4.5 shows changes in starch, sugars concentrations, and the ratio of starch and sugar as a function of stages of maturity in banana. At the onset of maturity there is little sugar in the fruit, and as maturity increases the fruit sweetness progressively increases due to hydrolysis of starch into sugars, particularly after stage 2, and starch practically disappears at stage 6.

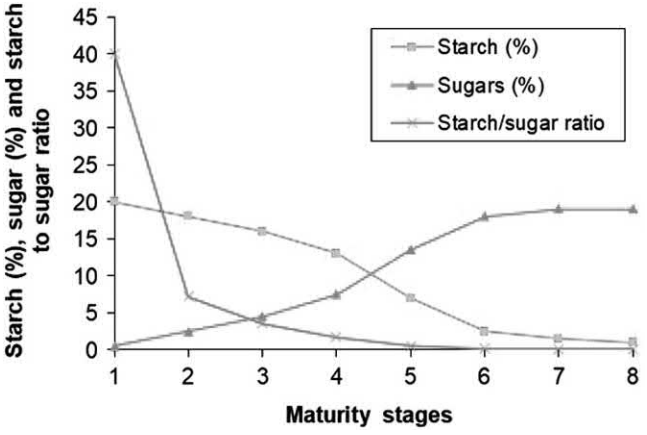

FIGURE 4.5 Relationship between the stages of maturity of banana and starch, sugar and starch–sugar ratio. (Data from Wills et al. [35].)

Every fruit has its unique assemblage of volatile compounds, and an increase in volatiles contributes to the flavor, taste, and aroma of the fruits. The main volatile compounds are acids, alcohols, esters, carbonyls, aldehydes, ketones, and hydrocarbons [12, 19, 35, 54]. These volatiles are present in extremely small quantities (<100 g/g fresh weight), and the total amount of carbon involved in their synthesis is less than 1% of that expelled as carbon dioxide [12]. Although ethylene does not have strong aroma and does not contribute to typical fruit aroma, it does influence the formation of volatiles in climacteric fruits. In both climacteric and non-climacteric fruits, the most important aroma volatiles compounds that increase during ripening are the esters. The characteristic or optimum flavor develops at a specific stage of the ripening process. In the case of tomatoes, while the color changes from the green to the red stage during ripening, the volatile concentration reaches a peak from the pink to red stages of maturity. In mango, the characteristic flavor develops only after the half-ripe stage (climacteric stage), and the extent of flavor generation depends on the temperature conditions during storage [54]. There is also a dramatic increase in aroma volatiles in non-climacteric fruits. Esters, acetals, alcohols, and aldehydes are formed in strawberry. In pineapple, there is a dramatic increase in ester production during ripening, while in citrus there are no changes in volatile production during storage. In grapefruit and pomelo, the changes in volatiles are very minor during storage, except for the important increase in nootkatone, which contributes significantly to pleasant grapefruit flavor.

Non-ethylene, non-respiratory organic volatiles, such as terpenes, carboxylic acids, alcohols, aldehydes, sulfur compounds, ammonia, and jasmonates, impact physiology and/or quality of fresh produce, particularly as antimicrobial or insecticidal agents [55]. Stress and injury, disease, cultivar, and atmospheric composition strongly influence their accumulation.

Fruits become softer upon ripening due to a series of changes at the cellular level. The composition, water content or turgor pressure, and cell wall constituents undergo transformation upon maturity [56–60]. The cellular structure weakens as a result of modification to the polysaccharides, such as cellulose, hemicellulose, and pectins, present in the cell wall and middle lamella by the action of several enzymes, such as polygacturonase and glycosidases. Polyuronide depolymerization, loss of galactan and arabinan, pectin demethylesterification and solubilization, depolymerization of matrix glycan, and cellulose depolymerization are responsible for degradative changes in the cell wall [56, 60]. Considerable variations exist among different fruits as shown in Table 4.9, which lists the extent of changes in arabinan, galactan, pectin solubilization, and depolymerization in the cell wall during

TABLE 4.9
Cell Wall Changes Responsible for Loss of Texture During Ripening

Cell Wall Change	Absent	Low	Moderate	High
Loss of arabinan	Watermelon Apricot Plum	Pepper Tomato	Avocado Peach Kiwifruit	Pear Blueberry
Loss of galactan	Cucumber Plum	Apricot Blueberry	Peach Apple Melon	Muskmelon Pepper Tomato
Solubilization of pectin	Watermelon Apple	Apple	Plum Strawberry Tomato Banana	Avocado Kiwifruit Blackberry
Depolymerization of pectin	Banana Strawberry Apple Pepper	Melon Papaya Watermelon	Peach Tomato	Avocado

Source: Brummell [60].

TABLE 4.10
Physiological Changes Induced by Phytohormones Other Than Ethylene

Physiological Changes	Phytohormone	Produce Affected
Sprouting	Abscisic acid, gibberellin, cytokinins, auxins	Onion
Senescence	Increased levels of gibberellin and low levels of auxins and cytokinins	Brussels sprouts, lettuce
Elongation of flower peduncle	Gibberellins	Cauliflower
Rooting	High levels of cytokinenins and low levels of gibberellins	Carrot
Pithiness of petiole	Abscisic acid	Celery
Ripening	Abscisic acid stimulates, auxins, gibberellins, and cytokinenins delay ripening	Tomato

Sources: Haard [43]; Salunkhe et al. [13].

ripening for some fruits [60]. These changes have an impact on the gel formation ability required for fruit preserves, juice yield and cloudiness of fruit juices, tissue collapse during thermal processing, etc.

4.5.4 Phytohormones Effects

Phytohormones play an important role in plants by controlling the growth and development processes of plant organs. Physiological processes influenced by hormones are ripening, rest, dormancy, rooting, sprouting, abscission, and floral induction [43, 61, 62]. Besides ethylene, the key hormones responsible for these processes are: auxins, gibberellin, cytokinins, abscisin (Table 4.10). The physiological response to these hormones has been mainly studied in relation to increasing the yield of produce and has been less focused on the postharvest effects [61, 62], since vegetables are harvested from different parts of the plant and stages of development, hence the physiological effects of hormones are, as expected, not limited to ripening and senescence as in the case of fruits.

Ethylene has been studied the most by postharvest physiologists. Ethylene is a natural product of plant metabolism and is produced by all tissues of higher plants and by some microorganisms [12, 32] and is also present in the atmosphere as a pollutant. Ethylene regulates many aspects of growth and development even at concentrations <0.1 ppm. The production of ethylene by fruits and vegetables varies substantially from <0.1 to >100 ml/kg h (Table 4.11). Climacteric fruits, generally, produce higher amounts of ethylene than non-climacteric fruits.

Ethylene is synthesized in plants from methionine, an amino acid, by a series of reactions by a highly regulated pathway (Figure 4.6). The key steps in this pathway are (i) conversion of methionine to S-adenosyl-l-methionine (SAM) by SAM hydrolase, (ii) SAM to 1-aminocycloproane-1-carboxylic acid (ACC) by ACC synthase, and (iii) ACC to ethylene by ACC oxidase [35, 53, 62]. Oxygen is required for ethylene biosynthesis while both O_2 and CO_2 are needed for its bioactivity [61]. ACC can also be converted into two conjugates, malonyl- ACC and -glutamyl-ACC by ACC-N-malonyltransferase and ACC--glutamyltransferase enzymes, respectively. Synthesis of these conjugates reduces availability

TABLE 4.11
Classification of Fruit and Vegetables According to Ethylene Production Rates

Class	Rate (ml/kg h)	Examples
Very low	<0.1	Artichoke, asparagus, cauliflower, cherry, strawberry, pomegranate, leafy vegetables, potatoes
Low	0.1–1.0	Blueberry, cranberry, cucumber, eggplant, okra, olive, pepper, persimmon, pineapple, pumpkin, raspberry, tamarillo, watermelon
Moderate	1.0–10.0	Banana, fig, guava, melon, honeydew, mango, plantain, tomatoes
High	10.0–100.0	Apple, apricot, avocado, cantaloupe, feijoa, kiwi, nectarine, papaya, peach, pear, plum
Very high	>100.0	Cherimoya, passionfruit, sapota

Source: Kader [28].

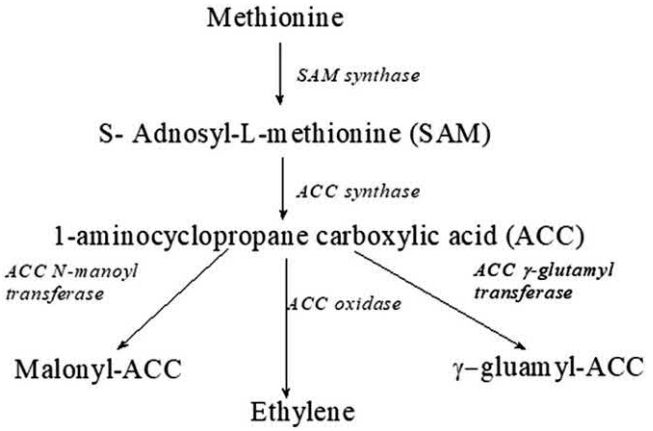

FIGURE 4.6 Biosynthetic pathway of ethylene production. (From King and Donohue [53]; Pech et al. [62].)

of ACC for conversion to ethylene. ACC synthase is the key enzyme in the pathway leading to the production of ethylene in plants. Only one of many genes controlling this enzyme may be responsible for the ripening action of ethylene. It may, therefore, be possible to control ethylene biosynthesis without influencing other physiological processes.

Ethylene stimulates ripening of climacteric and some nonclimacteric fruits, synthesis of anthocyanins, degradation of chlorophyll (degreening), germination of seeds, formation of adventitious roots, abscission and senescence, flower initiation, and respiratory and phenyl propanoid metabolism [63]. It also is known to accelerate its own synthesis in ripening climacteric fruits. Ethylene is used as a "ripening hormone" for climacteric fruits such as banana and mango commercially and as a "degreening hormone" for citrus fruits. The beneficial and adverse effects of ethylene depend on several factors such as the type of produce, cultivar, and the maturity at the time of harvest, temperature, and activity of other hormones, etc. Table 4.12 lists the adverse effects of ethylene on selected fruits and vegetables. Controlling the action of ethylene is of great commercial significance in fruits and vegetables as the adverse effects can lead to serious economic losses. Ethylene production rates by fresh fruits can be reduced by storage at low temperatures, by reduced oxygen (less than 8%, and/or elevated carbon dioxide above 1%), avoiding stressors, such as fruit injury, diseases incidence, and water stress, and cooling to reduce the rate of respiration [12, 32]. Saltveit [63] describes three main ways to control the action of ethylene by: preventing tissue exposure, its perception, and its response to ethylene. Tissue exposure can be avoided by excluding ethylene from the surrounding environment by scrubbing, inhibiting synthesis, and proper ventilation. Methods that work by blocking the perception of ethylene are: use of CO_2, silver, 1-methyl cyclopropene (MCP); reduction in temperature; and using ethylene-insensitive cultivars. MCP is being actively researched and now has been approved for use in some fruits in many countries [20, 64, 65]. The current model of its action suggests that MCP has ten times more affinity to the ethylene receptors than ethylene. This results in the beneficial effects of application of MCP in controlling ripening and senescence of fruits and vegetables.

TABLE 4.12
Adverse Effects of Ethylene on Fruits and Vegetables

Produce	Symptoms
Asparagus	Woodiness
Carrots	Bitterness due to isocoumarine formation
Potatoes	Sprouting
Lettuce	Russet spotting
Broccoli	Yellowing, abscission, off flavors
Eggplant	Browning of flesh and seeds, decay induction
Cucumber	Yellowing, softening
Sweet potatoes	Browning of pulp, off flavor, failure to soften upon cooking

4.5.5 Physiological Disorders and Breakdowns

Physiological disorders result from metabolic disturbances caused by a host of internal (nutritional imbalance) and external (temperature and surrounding atmosphere) factors. Table 4.13 lists some of the common disorders associated with common fruits and vegetables. Besides inducing serious loss of quality in terms of color, flavor, texture, and appearance, physiological disorders can predispose the produce to further deterioration by enzymes and microorganisms. When only superficial tissues are affected by the disorder, fruit may still be able to be processed without seriously impacting the quality of processed products. Maturity at harvest, cultural practices, and climate during growing season, produce size, and harvesting and handling practices can be involved in inducing disorders [25, 66, 67]. Preharvest factors that affect the incidence of postharvest disorders are: type of the produce, variety, nutritional status of produce and soil, level of maturity, temperature, fruit size and color, moisture content of soil, position of fruit on the tree, etc. Crop load and fruiting position are linked with bitter pit [66, 67] in apples and mealiness and browning of stone fruits [68]. Detailed information about the physiological disorders and diseases are covered in Refs. [69–71].

4.5.5.1 Disorders Due to Mineral Deficiencies

Plants require a balanced mineral intake from the soil and a favorable environment for proper development. Bitter pit in apples, characterized by brown lesions in flesh, dark and corky tissue below the skin, and slight bitter taste, develops due to preharvest low fruit Ca and high levels of K and magnesium. Pre- and postharvest application of Ca can control this disorder. Calcium nutrition together with maturity influences early development of water core in apples [72]. Fruit softening in papaya [73] and blossom end rotting of tomato have also been linked to low Ca levels [74]. Ca deficiency in vegetables leads to black-heart in celery, tip burn in chervil and Chinese cabbage, and blossom end rot in tomato and cucumber [75].

TABLE 4.13
Physiological Disorders of Selected Fruits and Vegetables

Fruit	Disorder	Common Symptoms	Cause
Apples	Sunscald or sunburn	White or yellow spots on the fruit facing sun followed by drying out and sunken areas	Heat stress to the tissue
	Storage scald	Irregular brown patches of dead skin developing during warming after cold storage	Chilling injury involving oxidation of α-farnesene to conjugated trienes
	Internal breakdown (brown heart)	Brown discoloration of flesh	CO_2 injury during storage
	Bitter pit	Brown lesion in flesh, dark and corky tissue below the skin	Low levels of calcium and high concentrations of potassium and magnesium, dissolution of middle lamellae by oxalic and succinic acid, changes in proton secretion and potassium permeability
	Water core	Water soaked regions in flesh and core	Inability of fruit to convert sorbitol to fructose and hence accumulation of sorbitol
Pear	Core breakdown	Brown, soft breakdown of core and surrounding tissues	Storing fruits beyond normal storage life
	Flesh spot decay	Partial browning of spots and/or development of cavities	Unknown
	Internal breakdown	Brown to dark brown water-soaked areas in core and/or flesh	Unknown
	Watery breakdown	Soft, watery deterioration	Slow cooling or exposure to warm temperatures
	Senescent scald	Brown to black discoloration of skin, loss of ripening capacity, adverse taste and odor	End of postharvest life and setting of fermentation
Pomegranates	Husk scald	Discoloration of husk	Oxidation of phenolics
	Chilling injury	Brown discoloration of skin, pitting, loss of red color of arils	Oxidation of phenolics in response to exposure to temperature <5°C
Stone fruits (apricots, peach, nectarine, plum, and prune)	Internal breakdown	Flesh browning, lack of juiciness due to mealiness or latheryness, accumulation of red pigmentation, black pit cavity, loss of flavor	Placing fruit at room temperature after cold storage during ripening Mealiness could be genetically controlled
	Inking, black stain	Brown and black spots on the skin	Abrasion damage in association with contamination with metals (iron, copper, aluminum)
	Surface pitting and bruising	Small sunken areas on skin and large flattened areas	Mechanical impact or compression
Tomatoes	Blossom drop	Fruit set failure	Prevalence of extreme temperature during pollination
	Blossom end rot	Brown to black spot on the blossom end	Calcium deficiency, excessive nitrogen fertilization
	Sun scald	Shiny white or yellow spots on the fruit facing sun followed by drying out and sunken areas	Fruit exposure to sun under extreme heat
	Fruit cracks	Radial and concentric cracks at the steam end	Heavy rainfall or irrigation after long dry period
	Catfacing	Puckering and scarring of the blossom end	Cloudy and cool weather during blooming
	Puffiness	Hollow inside and empty seed cavity	Defective pollination due to extremes of temperature, excessive nitrogen fertilization, and heavy rains

Sources: Crisosto [69]; Willis and Golding [35].

TABLE 4.14
Classification of Fruits According to Their Climate Area of Growth

Climate Zone	Examples
Temperate	Pome fruits (apple, pear, quince), stone fruits (apricot, cherry, nectarine, peach, plum), small fruits and berries (grape, strawberry, raspberry, blueberry, blackberry, cranberry)
Subtropical	Citrus fruits (grapefruit, lemon, lime, orange, pummelo, tangerine, and mandarin), non-citrus fruits (avocado, cherimoya, fig, kiwifruit, olive, pomegranate)
Tropical	Banana, mango, papaya, pineapple, carambola, cashew apple, durian, guava, longan, lychee, mangosteen, passion fruit, rambutan, sapota, tamarind

Source: Kader [12].

4.5.5.2 Disorders Due to Environmental Factors

4.5.5.2.1 Low Temperature Injuries or Disorders

Chilling and freezing injuries result from exposure of plant tissues to low temperatures. Both types of defects are prevalent in fruits and vegetables of tropical and subtropical origin, which lack the ability to adapt to low temperature environments. Table 4.14 classifies fruits according to climate area of growth and can indicate fruits susceptible to low temperature injury. The susceptible produce tend to have low storage potential as very low temperatures cannot be used during storage, transport, and handling. The lowest safe storage temperature has to be well above the chilling injury (CI) threshold of susceptible product. The CI manifests as symptoms such as pitting, browning, scalded appearance, darkening of the skin, changes in the flavor and texture, and the loss of ripening ability. The severity of the symptoms depends on the type of the produce, and time and temperature of the exposure. The mechanism of development of chilling injury is complex. Changes in the membrane lipids and dissociation of enzymes and other proteins have been proposed to be the likely causes of development of CI [35, 76, 77]. Most tropical and subtropical produce show CI upon exposure to chilling temperatures below 10–15°C [77]. Table 4.15 shows CI symptoms and the temperature at which injury symptoms appear for selected fruits and vegetables. Internal discoloration, water-soaked appearance, and increased susceptibility to plant pathogens result in the loss of quality for both fresh and processed markets. Besides, avoiding exposures to injury temperatures, CI can be minimized by reducing the exposure time to injury temperatures, precooling produce in stages to build adaptation, selection of resistant varieties, selecting fruits at the appropriate level of ripening, intermittent warming [35, 77, 78] and cooling treatments, controlled atmosphere storage (>2% CO_2 and <5% O_2), maintaining high storage relative humidity, and improving calcium nutrition [35, 39, 69, 76, 77].

Freezing injury (FI) results from exposure of plant tissues to freezing temperatures (<0°C) and formation of ice crystals, which damage the cells, mostly irreversibly. The injury initiates osmotic stress and damage to the cell membrane responsible for water loss and death of cells. The factors that affect FI are type of produce, variety, nature of solutes, field temperature, etc. [35, 69, 77]. Susceptibility of fruits and vegetables to FI varies between the produce (Table 4.16). Light freezing damages the most susceptible crops, while the least susceptible products can be frozen several times before injury occurs. Moderately susceptible produce can recover from one or two episodes or freezing. Susceptibility to FI, however, does not depend on the freezing point, which may vary between –0.1 to –1.8°C [77]. Warm season crops are more highly susceptible to FI than those that are grown in the cold season. The symptoms of the FI include discoloration of the tissue, water-soaking appearance, blistering, and pitting. The damaged tissues are prone to further decay by microorganisms and mechanical injuries, particularly bruising.

4.5.5.2.2 High Temperature Injures/Disorders

Exposure of tissues to high temperature during production or postharvest results in injuries or disorders, which may cause loss of ability to ripening normally, burnt or scorched peal, and darkening of the pulp. Examples of such disorders are scald in apples and tomatoes and blossom drop in tomatoes. Besides avoiding long exposure to sun, superficial scald in apples can be controlled by postharvest spray with ethoxyquin and/or diphenylamine [13]. The latter is more effective.

4.5.5.2.3 Injuries/Disorders Due to Exposure to Adverse Atmospheres

Exposure to low levels of O_2 and/or high levels of CO_2 may lead to tissue injuries such as internal browning in apples and pears when the tissue tolerance is exceeded. The factors that influence the susceptibility are variety, low crop load, exposure to higher concentration of CO_2 at the time of harvest and presence of coatings that restrict the diffusion of gases [68].

4.5.6 Other Biochemical Changes

Chemical and enzymatic changes cause tissue softening, off flavors, pigment loss and off-colors, and overall decline in nutritional value and taste [27]. The enzymes that catalyze biochemical reactions responsible for these changes are given in Table 4.17. Softening of the tissues can be due to hydrolysis of starch and cellulose by amylases and cellulose enzymes, respectively, and degradation of pectin by pectinases. There are three key enzymes involved in the degradation of pectins, endo- and exopectate hydrolases and pectin esterase. Endopectate hydrolase yields methyl galacturonide oligomer, and exopectate hydrolase acts on demethylated pectin produced by pectin esterase to produce galacturonic acid.

TABLE 4.15
Chilling Injury of Fruit and Vegetables Stored above Freezing Temperatures

Class	Produce	T_{inj}[a]°C	Symptoms
A (0–5°C)	Apples (some cultivars)	2–3	Internal browning, brown core, soggy tissues and soft scald
	Asparagus	0–2	Dull, grey-green, limp tips
	Avocados	4.5–13	Grayish brown discoloration of flash
	Lima beans	1–4.5	Rusty brown specks, spots, or areas
	Cranberries	2	Rubbery texture, red flash
	Guavas	4.5	Pulp injury, decay
	Cantaloupes	2–5	Pitting, surface decay
	Watermelon	4.5	Pitting, objectionable flavor
	Oranges	3	Pitting and brown stain
	Pomegranate	4.5	Pitting, external and internal browning
	Potatoes	3	Mahogany browning, sweetening
	Tamarillos	3–4	Surface pitting and discoloration
B (6–10°C)	Snap beans	7	Pitting and russeting
	Cucumber	7	Pitting, water-soaked spots, and decay
	Eggplant	7	Surface scald, Alternaria rot, blackening of seeds
	Limes	7–9	Pitting, turning tan with time
	Honeydew melon	7–10	Reddish tan discoloration, pitting, surface decay, failure to ripen
	Casaba, Crenshaw, and Persian melons	7–10	Pitting, surface decay, failure to ripen
	Okra	7	Discoloration, water-soaked areas, pitting, decay
	Fresh olives	7	Internal browning
	Papaya	7	Pitting, failure to ripen, off flavor, decay
	Sweet peppers	7	Sheet pitting, Alternaria rot on pods and calyxes, darkening of seed
	Pineapple	7–10	Dull green when ripened
	Pumpkin (hardshell and squashes)	10	Decay, especially Alternaria rot
	Tomatoes (ripe)	7–10	Water soaking
C (11–20°C)	Banana (green or ripe)	11.5–13	Dull color when ripened
	Grapefruits	10	Scald, pitting, watery breakdown
	Jicama	13–18	Pitting, membranous staining, red blotch
	Mangoes	10–13	Grayish scald like discoloration of skin, uneven ripening
	Sweet potatoes	13	Decay, pitting, internal discoloration, hard core when cooked
	Tomatoes	13	Poor color when ripe, Alternaria rot

Source: Wang [77].
[a] Approximate lowest safe temperature.

Formation of galacturonide oligomer and galacturonic acid leads to softening of plant tissues. However, formation of calcium pectate as a result of addition of calcium has a firming effect on the tissue. The activity of pectin-degrading enzymes seriously impacts the gel-forming ability of fruits fundamental to the production of fruit preserves.

Fruits and vegetables contain varieties of phenolic compounds that participate in browning reactions catalyzed by enzymes. Cutting or injuring plant tissue results in browning of the cut surface due to enzymatic oxidation of phenolic compounds due to cell de-compartmentation of substrate and enzymes. In the presence of O_2, phenolases oxidize phenols to benzoquinones, which, although colorless, are further converted into brown pigments in fruits and vegetables (potato, mushroom, apple, and banana). While the physiological role of these enzymes in plant cells is not very well understood, these are produced by plants in response to environmental stress. These compounds have strong antioxidant activity. The factors influencing enzymatic browning are type and content of phenolic substances, enzymes, temperature, and presence of

TABLE 4.16
Relative Susceptibility of Fruits and Vegetables to Freezing Injury

Highly Susceptible	Moderately Susceptible	Slightly Susceptible
Vegetables		
Artichoke	Cabbage	Beetroots
Asparagus	Carrot	Brussels sprouts
Beet	Cauliflower	Celeriac
Broccoli	Chives	Collard
Celery	Endive	Horseradish
Cucumber	Leek	Kale
Eggplant	Onion (bulb)	Kohlrabi
Sweetcorn	Onion (green)	Parsnip
Lettuce	Parsley	Rutabagas
Okra	Shelled peas	Salsify roots
Sweet pepper	Peas (pod)	Turnip roots
Potatoes	Radish	
Summer squash	Spinach	
Sweet potatoes	Winter squash	
Tomatoes		
Fruits		
Apricot		
Avocado	Apples	Dates
Banana	Cranberries	
Berries (except cranberries)	Grapes	
Lemon	Oranges	
Limes	Pears	
Peaches		
Plums		

Source: Wang [77].

TABLE 4.17
Enzymes Responsible for Key Reactions Associated with Ripening and Senescence in Fruits and Vegetables

Enzyme	Reaction	Result
Polyphenoloxidase, catalase, and peroxidase	Oxidation of phenolics	Formation of precursors to colored polymers leading to undesirable browning
Polygalacturonase	Hydrolysis of glycosidic bonds between adjacent polygalacturonic acid residues in pectin	Tissue softening
Pectin esterase	Hydrolysis of ester bonds of galacturonans in pectin	Tissue firming
Lipoxigenase	Oxidation of lipids	Production of off flavor and off odors
Ascorbic acid oxidase	Oxidation of ascorbic acid	Loss of nutrition quality
Chlorophyllase	Cleavage of phytol ring from chlorophyll	Loss of green color
Amylases	Hydrolysis of amylose and amylopectin	Loss of texture and increase in sweetness due to production of sugars
Cellulase and hemicellulases	Hydrolysis of cell wall	Loss of texture
Proteases	Hydrolysis of proteins	Loss of nutritional value and increase or decrease in digestibility
Lipase	Hydrolysis of lipids	Hydrolytic rancidity
Phytase	Hydrolysis of phytic acid	Liberation of phosphates
Glucose oxidase	Oxidation of glucose	Formation of hydrogen peroxide

inhibitors. Chlorogenic acid, a phenol found in potatoes, is also responsible for after-cooking blackening of potatoes. The reaction involves formation of ferric-dichlorogenate from chlorogenic acid and ferrous ions [58]. Conditions leading to a higher ratio of chlorogenic acid to citric acid are conducive to after-cooking blackening. Postharvest sweetening of potatoes is a serious problem in cold-stored potatoes due to conversion of starch to sugars, which can lead to non-enzymatic browning in potato chips and can be avoided by warming the potatoes before use. Injuries can also lead to the formation of stress metabolites through linoleic/linolenic acid cascade producing traumatin and jasmonic acid, which have a role in forming a defense system against insects and microorganisms. Degradation of lipids by lipase and lipoxigenase enzymes can lead to the formation of off flavors in fruits and vegetables (e.g. beans and peas) and can be avoided by inactivating these enzymes or by knocking the genes responsible for their expression.

4.6 CONCLUSION

Fruits and vegetables are live tissues, which have unique postharvest characteristics that influence the postharvest quality of both fresh and processed products. In this chapter, a brief overview of postharvest quality and its relationship to physiological processes is provided. Data on postharvest physiological processes such as respiration, maturity and senescence, and transpiration should be useful to both the fresh and processed food industries in providing the ideal quality required by the modern consumer.

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5 Postharvest Handling and Treatments of Fruits and Vegetables

V. K. Mishra and T. V. Gamage

CONTENTS

5.1 INTRODUCTION

The quality of fruits and vegetables deteriorates progressively after harvest within a short time due to a series of physical, physiological, and pathological agents the produce is exposed to before reaching a consumer or a processor. Handling operations vary according to the produce. Figure 5.1 depicts a generic postharvest value chain with common handling operations. Both quantitative (reduction in weight and wastage due to biotic factors) and qualitative (reduction in color, flavor, and texture) losses anywhere along the chain occur in the field, during packaging, storage, distribution, and transportation. Postharvest management practices facilitate the continuous supply of fruits and vegetables to fresh, minimally processed, and processed markets. Given the distance between the sites of production and consumption, these perishable commodities need to travel a long distance, and hence the maintenance of quality over the entire value chain is an onerous task. Generally, fruit and vegetable processing is very seasonal in nature, and the harvested produce must be quickly processed to avoid losses. The processor must assure that all the quality attributes at the time of harvest are maintained before processing. Normally, quality at harvest can only be maintained and not improved down the value chain except for climacteric fruits, which can be ripened after harvest to achieve ideal eating quality.

A good quality processed product can only be possible when good quality raw materials are used in its manufacture. Only those cultivars that are suitable for a particular process application are procured. Cultivars suitable for table-fresh markets are very different from those which are suited for processing. Generally, the produce is harvested at the stage when the eating quality is at its peak as this commands maximum market revenue. Quality specifications vary according to the raw material required for a given processing application and often include optimum color, texture, and flavor; freedom from pathogens and spoilage microorganisms including their metabolic products; freedom from toxic residues such as

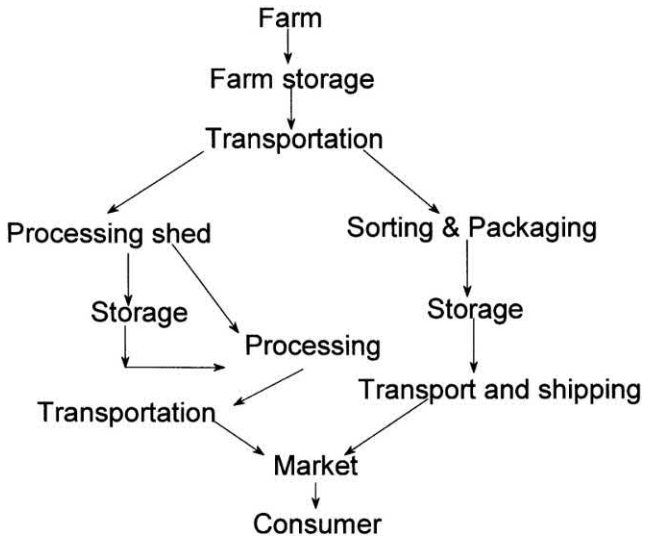

FIGURE 5.1 Postharvest value chain for fruit and vegetables.

pesticides; reasonable storage life, high nutritive value; and extended availability. Table 5.1 lists the quality specifications requirements for various fruit and vegetables processed into various products.

5.2 POSTHARVEST HANDLING OPERATIONS

5.2.1 Sorting and Grading

Most fruits and vegetables are sorted and graded for marketing and these processes have a role in protecting and enhancing product quality. These are generally an important part of field or packinghouse operations and help in reducing cross-contamination of healthy stock destined for storage, transport, distribution, marketing, and processing, in addition to facilitating ease in trade. The produce is sorted according to size, shape, color, and appearance after passing the minimum requirements of quality. The damaged and immature fruits must be removed, as these might become sources of ethylene gas, which will increase the rate of respiration, ripening, and senescence of healthy produce.

Grading determines whether the product meets a specific quality standard prescribed by the local or international market, separates products into different quality grades to determine the price paid to the farmers or to determine the sale value, and enables removal of off-grade products. Grade standards and specifications vary between and within countries, and local authorities should be consulted for updates. Optimal maturity, color, sugar, solids, moisture content, size, and absence of defects are some of the factors considered for various fresh market products. The domestic marketing orders of the USDA specifies mandatory grades for avocados, Irish potatoes, limes, filberts, raisins, onions, table grapes, walnuts, kiwifruit, dates, prunes, canned ripe olives, grapefruits, tomatoes, and oranges [1].

Traditionally sorting and grading are done by hand, which is extremely labor-intensive. Labor shortages and a lack of overall consistency have driven the search for automation of this operation. Color and shape are sorting parameters used in the implementation of many automated vision systems, which involve image acquisition and processing. Optical sorting is currently being used for apples destined for canning in Australia. Magnetic resonance spectroscopy and imaging, near infrared spectroscopy, acoustic response, and impact response are being investigated for their utility in automated sorting and grading of produce. Equipment used in dumping, conveying, and grasping during sorting and grading should have smooth and properly cushioned surfaces to avoid injury.

TABLE 5.1
Raw Material Quality Specifications for Processed Fruit and Vegetables

Processed Products	Raw Materials	Quality Specifications
Fruit juices	Citrus, apple, tomatoes	Acidity, sugar content, flavor
Chips and fries	Potatoes, banana, taro	Texture, starch content, and reducing sugars
Canned products	Apples, peach, pear	Color, texture, flavor
Preserves	Various fruits: apples, peaches	Sugar, pectin content, acidity
Pickles	Cucumber, olive, cabbage	Composition, sugar content, texture
Concentrates: sauce, puree	Tomatoes, apple	Total solids
Alcoholic beverages	Grapes, apples	Fermentable sugar, acidity
Dried products	Mango, apricot	Composition, solid content
Frozen products	Peas, carrots, onions	Composition, color, texture, flavor

5.2.2 Packaging

The packaging contributes greatly to efficient marketing of fruits and vegetables as it (i) serves as an efficient handling unit, (ii) provides a convenient warehouse or home storage unit, (iii) protects quality and reduces waste by avoiding mechanical damage, reducing moisture loss, providing a beneficial modified atmosphere, providing clean or sanitary produce, preventing pilferage, (iv) provides service and sales motivation, (v) reduces cost of transport and marketing, and (vi) facilitates the use of new modes of transportation [2].

Two types of packaging are common in the fresh produce trade. Large-sized containers are used for transport and wholesale, and small-size packagers for retail trade. Properly designed containers for transporting and marketing can maintain product freshness, succulence, and quality by significantly reducing mechanical damage during handling, transport, and storage. The container must be strong enough to withstand stacking and impact of loading and unloading, without bruising or scarring the produce. Thus, containers may require the use of liners, pads, trays, or tissue wraps to

prevent damage from contact with rough surfaces or adjacent produce. The produce can be packed in box (wooden or paper) with absorbent, lining, or padding materials or in bags. The choice of packaging material is based on the requirements of stacking height, duration of storage, pretreatments, cooling, and cost. Lug and pallet boxes are also used. Pallet boxes are used for bulk handling, which saves loading and unloading time, and manual labor [3].

5.2.2.1 Types of Damage

The fresh produce is mechanically or manually handled several times on packing lines before it arrives at the point of consumer purchase. Mechanical damage occurs in the postharvest handling system primarily by impact and compressive forces. The compressive forces act on the product when it is handled in bulk and are normally static loads (in bins, stacks) or dynamic compressive loads (bin handling and transport). The excessive impacts occur during harvesting, grading, handling, and transportation, and excessive compression loads occur during bulk and package handling.

Bruising results from fruit hitting each other during transportation and handling, and from contact with the hard surfaces of machinery, the container, or other handling equipment. Bruising can be caused by intermittent shocks, compressive forces, or prolonged low-level vibrations occurring during transportation of produce from the orchard or field to the packing house, and from the packing house to the retail store [4]. Bruising causes enzymatic browning in apples, pears, peaches, apricots, grapes, and bananas.

Compression damage is the primary cause of damage to fruit while it is handled in bulk. The force on the product is transferred from the vehicle transporting the bin to the produce. The energy is dissipated through movement of the product and absorption by the produce. The severity of the levels of bruising resulting from bulk handling has been reported in various studies. The forces vary considerably within bins according to the load paths due to the produce stacking pattern. This loading pattern is also influenced by the bin design and the transport method [4].

5.2.2.2 Cushioning and Other Protections

Packing line equipment and other harvesting and postharvest handling equipment are traditionally designed and installed using many transfers from one operation to the next. During this handling the produce hits (impacts) hard surfaces or other produce. Cushioning and velocity control devices can avoid bruising in handling systems. A cushioning material must provide effective energy absorption and dissipation and not create the critical stress/strain level in the produce tissue that will initiate bruising. The packing line should be designed with an appropriate drop height or roll velocity. If hard surfaces on the equipment are adequately cushioned, and the roll velocity of each item is controlled to a low enough level, impact bruises can be avoided.

Immobilization and proper cushioning of the produce help in reducing damage due to cuts, punctures, bruises, abrasion, impact, and friction. This is done by the use of various types of trays, or by certain volume fill techniques, such as padding or cushioning [3]. The material used for padding should (i) have the ability to absorb the impact energy without damaging the produce, (ii) not impart a high rebound energy to the produce, (iii) have durability to internal structure fatigue and surface wear (needs lower thickness), (v) cushion cleanup, sanitation, and compatibility with water, fungicides, waxes, and cleaning solutions must be excellent, (vi) cushion physical properties (thickness, stiffness). The materials that can be used are PVC, polyethylene, neoprene, polyurethane, wool carpet, polypropylene, and poron. The materials are usually made with a porous internal structure and specific surface characteristics [4]. Commonly used padding materials are leaves, straw, grass, coconut husk, paper, and plastics.

Plastic films, mesh, or net, plastic-lined paper may also be used to prepackage fresh produce. Individual seal packaging or uni-packaging creates a water-saturated atmosphere around the fruit and reduces water loss and shrinkage. The advantages are that (i) it may be an alternative to expensive traditional refrigeration and sophisticated controlled atmosphere storage, (ii) it doubles and sometimes triples the shelf life, (iii) it also delays physiological deterioration better than when only cooling alone is used, (iv) it may reduce chilling injury in some fruits, such as in citrus. Films used in packages may be used as carriers of fungicides to reduce toxic residue in products and/or ethylene absorbing substances to delay ripening. The use of perforated films allows optimum gas exchange and avoids the accumulation of ethylene in the enclosed micro-atmosphere [6]. Perforated films are more suited to high O_2-demand produce. The limitation of using seal packaging is the possibility of development of off-flavors caused by poor gas exchange and enhancement of decay and spoilage due to the phytotoxic micro-atmosphere (low oxygen, excessive carbon dioxide and ethylene).

5.2.3 Transportation

The harvested produce is transported to the packing and processing sheds inland via road by trucks in pallet boxes. Bulk trucks are used for fruit such as oranges and vegetables. Overseas transportation is normally by sea and rarely by air. The proper management of temperature, humidity, and ventilation is the main requirement. Bruised, decayed, and overripe products are sorted out before transportation to avoid dissemination of diseases, induction of ethylene gas, increase in respiration, and evolution of heat and loss of water. When large-sized products, such as watermelons, muskmelons, pumpkins, yams, and cabbages, are transported in bulk using trucks, trolleys, or lorries, products should be carefully stacked and adequately covered to protect from the environment.

Refrigeration during transportation is the most effective means of reducing losses. Proper insulation and ventilation of trucks help in minimizing loss of quality. The following measures have been suggested to minimize heat accumulation during the transportation of fruit and vegetables [7, 8]: (i) avoiding closed vehicles without refrigeration except for local

deliveries, (ii) fitting open-sided or half-boarded trucks with roofing and siding to protect produce from direct sun and wind exposures; (iii) fitting a second white painted roof 8–10 cm above the main roof to act as radiation shield; (iv) provision for air intake in conjunction with louvers in unrefrigerated vehicles used in long-distance transport to ensure positive airflow through the load; and (iv) equipping transport vehicles such as trucks, rail cars, and sea containers with refrigeration for long journeys. Another important consideration is to make sure that only compatible fruits and vegetables are transported together. Table 5.2 provides the listing of commodities that are compatible during transportation and storage.

5.2.4 Precooling

Good temperature management throughout the postharvest chain is key to avoiding postharvest losses and to the preservation of quality. Rapid cooling of the produce to a safe storage and transportation temperature is imperative in the preservation of quality and to increase the shelf life by arresting the deteriorative changes caused by physiological and pathological agencies. The harvested produce contains a substantial amount of heat associated with the product temperature which is known as field heat, a significant part of cooling load. Precooling is the rapid extraction of heat from the product before transport, storage, and processing. Depending on the temperature and type of fruit or vegetable, the product will lose its quality in a short time unless promptly and appropriately cooled. Precooling assists in reducing the rates of metabolic activity, such as respiration, transpiration, and ethylene production, minimizing growth of decay microorganisms, and easing the load on the cooling system downstream for storage. Improved flexibility in marketing is an additional benefit.

The amount of field heat necessary to be removed depends on the produce and the required storage temperatures. At the time of harvest, the produce temperature is the same as that of the environment; wherever possible, the produce must be harvested when the ambient temperature is low, during night, morning, or evening, to avoid high cooling loads. The amount of heat to be removed can be estimated by methods described in several publications [9–11] or estimated from the compositional data [12].

TABLE 5.2
Compatibility Groups for Transport and Storage of Fresh Fruit and Vegetables

Group	Temperature (°C)	RH (%)	Commodity
1	0–2	90–95	Apples, apricots, beets (topped), berries (except cranberries), cashew apples, cherries, coconuts, figs (not with apples), grapes (without sulfur dioxide), horseradish, kohlrabi, leeks, longan, loquat, lychee, mushrooms, nectarines, oranges,[a] parsnips, peaches, pears, persimmons, plums, pomegranates, prunes, quinces, radishes, rutabagas, turnips
2	0.2	95–100	Amaranth,[b] anise,[b] artichokes,[b] asparagus, bean sprouts, beets,[b] Belgian endive, berries (except cranberries), bok choy, broccoli,[b] Brussels sprouts,[b] cabbage,[b] carrots,[b] cauliflower, celeriac,[b] celery,[b] cherries, corn (sweet),[b] daikon,[b] endive,[b] escarole,[b] grapes (without sulfur dioxide), horseradish, Jerusalem artichoke, kiwi fruit, kohlrabi,[b] leafy greens, leeks[b] (not with figs or grapes), lettuce, lo bok, mushrooms, onions[b] (green not with figs, grapes, mushroom, rhubarb or corn), parsley,[b] parsnip,[b] peas,[b] pomegranate, raddichio, radishes,[b] rhubarb, rutabagas,[b] salsify, scorzonera, snow peas, spinach, turnips,[b] water chestnut, watercress
3	0.2	65–75	Garlic, onions (dry)
4	4.5	90–95	Cactus leaves, cactus pears, caimito, cantaloupes,[b] clementines, cranberries, lemons,[a] lychees, kumquat, mandarin,[a] oranges (California and Arizona), pepino tamarillo, tangelos,[a] tangerines,[a] ugli fruit,[a] yucca root
5	10	85–90	Beans, calamondin, chayote, cucumber, eggplant, haricot vert, kiwano, malanga, okra, olive, peppers, potatoes, pummelo, squash (summer and soft shell), tamarind, taro root
6	13–16	85–95	Atemoya, avocados, babaco, bananas, bitter melon, black sapote, boniato, breadfruit, canistel, carambola, cherimoya, coconuts, feijoa, ginger root, granadilla, grapefruit, guava, jaboticaba, jackfruit, langsat, lemons,[a] limes,[a] mammy, mangoes, mangoseen, melons (except cantaloupes), papaya, passionfruit, pineapple, plantain, potatoes (new), pumpkin, rambutan, santol, soursop, sugar apple, squash (winter, hard shell), tomatillos, tomatoes (ripe)
7	18–21	85–90	Jicama, pears (for ripening) sweet potatoes,[c] tomatoes (mature green), watermelon,[c] white sapote, yams[c]

Source: McGregor [8].

Group 1: Many products produce ethylene.

Group 2: Many products are sensitive to ethylene.

Group 3: Moisture damages these products.

Groups 4 and 5: Many products are sensitive to ethylene and/or chilling injury.

[a] Citrus fruits treated with biphenyl may give odors to other products.

[b] Can be top iced.

[c] Separate from pears and tomatoes due to ethylene sensitivity.

5.2.4.1 Methods of Precooling

Precooling can be accomplished by simply blowing cold ambient air over the produce; however, refrigeration is required for ensuring the short cooling time that is critical in preventing the loss of quality. Cooling rates depend on the type of product, its size, weight, and the surface-area-to-volume ratio. A small-sized product with a large surface-area-to-volume ratio cools at a faster rate. Cooling rates are often expressed as several half-cooling times and can be used for comparing and predicting the effectiveness of different cooling methods for a given cooling time interval irrespective of the temperature of the produce or cooling medium used. Half time is the time required to reduce the temperature difference between the product and the cooling medium by one-half. Figure 5.2 shows a typical cooling curve for a product being cooled from an initial temperature of 30°C by air maintained at 2°C. As depicted in the figure the rate of cooling slows down as cooling progresses. The curve shows three half-cooling periods corresponding to one-half, three-quarters, and seven/eighths cooling, which is generally considered adequate for the transport and storage of most commodities. The essential information required for the design and operation of coolers is available in several publications [9–15]. The most commonly used methods of precooling are described in the following sections.

5.2.4.1.1 Room Cooling

This method involves using cold air as a medium to extract heat from the produce. High relative humidity (90–95%) is maintained in the air to avoid desiccation and weight loss during cooling. The method is not recommended for produce packed in bulk. For efficient heat removal, the produce container should be well-vented and stacked so that the container surface is in contact with cold air and the storage space is utilized to the maximum extent. Since the rate of heat removal by still air is slow it takes longer to cool produce to a safe transit or storage temperature.

5.2.4.1.2 Forced Air or Pressure Cooling

This is a modification of room cooling where cold air is forced through the produce containers and around the produce to speed up cooling to reduce cooling time to 10–25% compared to room cooling. Fans specially positioned in the room create the pressure differential to circulate air. Cooling time depends on the speed of the airflow provided sufficient refrigeration capacity is available for a given duty. The problems of moisture condensation on the produce, package, and the wall are eliminated due to air movement. Forced air cooling is accomplished by using three systems: cold wall, forced air tunnel, and serpentine cooling.

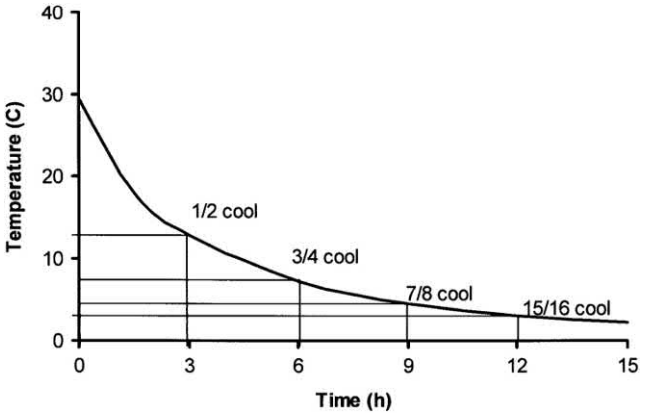

FIGURE 5.2 Typical cooling curve showing half cooling times for fruit and vegetables.

5.2.4.1.3 Package Icing

This method involves keeping finely crushed, flaked ice or an ice–water mixture in direct contact with the produce for cooling and maintaining low temperatures during short-term storage, transit, and display in superstores. The latent heat of melting of ice (334 kJ/kg) provides the cooling effect. The method is limited to products that can tolerate both the weight of the ice and water that wets the product and the package. It is commonly used for cooling spinach and broccoli during transport and retail displays. Use of ice slurry is recommended to avoid mechanical damage due to sharp end ice.

5.2.4.1.4 Hydrocooling

Hydrocooling is a rapid way to cool large batches of product by spraying or flooding the commodity with near-freezing water (~0°C). Near-freezing water cools product about 15 times faster than air, allowing for greater harvesting and marketing flexibility. While there is no desiccation of the product, water can be a source of contamination if soil and debris picked up during cooling are not removed before recycling. Water needs to be appropriately filtered and disinfected. Chlorination is used to disinfect water at 50–200 ppm. Hydrocooling is best suited to medium- to large-scale cooling of product and packages that are both water- and chlorine-tolerant.

5.2.4.1.5 Vacuum Cooling

The cooling effect is generated by partial evaporation of moisture directly from the product, which is placed in a chamber, and a vacuum is drawn to <1 kPa from atmospheric pressure. Evaporation results from a reduction in the vapor pressure of water under vacuum. This method does not require any cooling medium and is relatively faster for compatible produce (leafy vegetables such as Asian greens, silverbeet, broccoli) (www.postharvest.net.au/postharvest-undamentals/cooling-and-storage/cooling-methods/). The reduction in product temperature for a unit percent weight loss can be determined by the ratio of the latent heat of vaporization and heat capacity of the produce. Wang and Sun [16] calculated a reduction of 6.5°C/1% weight loss for leafy vegetables containing 90% moisture with a corresponding shelf life of 2.5 days at 12°C for head lettuce, which were cooled for 20 mins, sealed in polypropylene film and stored for a week at 2°C. This method is suitable for products that have a high surface-to-mass ratio, freely available water, are highly porous, and whose structure will not be damaged by the removal of water. The product may incur a weight loss of about 3–4%; however, adding a

preselected amount of water before or during pre-cooling can prevent weight loss.

5.2.4.1.6 Evaporative Cooling

This is a relatively inexpensive method suitable for precooling produce that requires relatively warmer storage temperatures, such as tomatoes and cucumber. The cooling effect results from the evaporation of water when dry air is blown over wet product. In the process of evaporation, water absorbs energy from dry air to reach its latent heat of vaporization (2260 kJ/kg) and change its phase from liquid to vapor. The method is suited to areas where low ambient humidity (<65% RH) air is readily available.

The choice of an appropriate cooling method is a very important decision that a grower or packinghouse needs to make. The decision is based on (i) the nature of the product to be cooled (e.g. chilling sensitivity), (ii) The temperature of the produce at the time of harvest, (iii) The cooling time required, (iv) The product throughput, (v) the type of packaging used, (vi) The desired storage life, and (vii) the comparative energy efficiency, availability, and associated capital and operating costs. Highly perishable fruits, such as strawberries, bush berries, and apricots, need be cooled to their optimum storage temperature (0°C) in a relatively shorter time (within 6 hours of harvest) than most other fruits (within 12 hours of harvest) [13]. Thompson [9] compared common methods of precooling based on cooling time, moisture loss, water contact with the product, potential for decay contamination, capital cost, energy efficiency, need for water-resistant packaging, portability, and feasibility of in-line cooling. Precooling methods suitable for selected fruit and vegetables are given in Table 5.3.

TABLE 5.3
Recommended Precooling Methods for Selected Fruit and Vegetables

Fruit or Vegetable	Precooling Methods Used
Asparagus	Hydro-cooling, package icing
Apples	Room, forced air, and hydro-cooling
Apricots	Room and hydro-cooling
Beans, snap	Room, forced air, and hydro-cooling
Broccoli	Package icing, forced air, and hydro-cooling
Brussels sprouts	Hydro-cooling, vacuum, and package icing
Cabbage	Room, forced air cooling
Cauliflower	Hydro-cooling and vacuum cooling
Cherries	Forced air and hydro-cooling
Cucumber	Forced air and hydro-cooling
Grapes	Forced air cooling
Lettuce	Hydro-cooling and package icing
Peas	Forced air and hydro-cooling
Potato	Forced air and hydro-cooling
Peaches	Forced air and hydro-cooling
Pears	Room, forced air, and hydro-cooling
Spinach	Hydro-cooling and package icing
Tomato	Room and forced air cooling

5.2.5 Storage and Distribution

Most horticultural produce has a short harvesting season, and short- and/or long-term storage is necessary to not only extend the marketing period for fresh produce but also to regulate the product flow and extend the processing season. The main objectives of storage are (i) to extend the availability of fresh produce in the market, (ii) to ensure the continuous supply of quality raw material to the processors, (iii) to extend the length of the processing season, (iv) to hold raw material obtained during favorable price situations, (v) to condition certain commodities, such as potatoes, onions, and garlic, (vi) to ripen certain fruits, such as mangoes and bananas [15].

Storage requirements vary among fruits and vegetables and can be classified into five groups [10] (Table 5.4) that are based on the rates of respiration and ethylene generation, and perishability. Storage life of <2 weeks to >16 weeks may be observed depending on the commodity. For example, green onions are highly perishable with <2 weeks of storage life compared to 8–10 weeks for dried onions. Variations do exist between and within fruit and vegetable and their cultivars. The storage potential of onions follows the order: yellow > red > white > Spanish and sweet [10]. Most vegetables, except root and tuber crops, are consumed fresh and do not require storage

TABLE 5.4
Storage Potential of Horticultural Produce in Air at Near-Optimum Storage Temperature and Relative Humidity

Class	Storage Life (weeks)	Degree of Perishability	Commodity
I	<2	Very high	Apricot, blackberry, blueberry, cherry, fig, raspberry, strawberry, asparagus, bean sprouts, broccoli, cauliflower, green onion, leaf lettuce, mushroom, muskmelon, pea, spinach, sweetcorn, tomato (ripe)
II	2–4	High	Avocado, banana, grape, guava, loquat, mandarin, mango, melons, nectarine, papaya, peach, plum, artichoke, green beans, Brussels sprouts, cabbage, celery, eggplant, head lettuce, okra, pepper, summer squash, tomato (partially ripe)
III	4–8	Moderate	Apples and pea (some cultivars), grape (sulfur dioxide treated), orange, grapefruits, lime, kiwi, persimmon, pomegranate, table beet, carrot, radish
IV	8–16	Low	Apple and pears (some cultivars), lemon, potato, dry onion, garlic, pumpkin, winter squash, sweet potatoes, taro, yam
V	>16	Very low	Tree nuts and dried fruits

Source: Kader [10].

for a significant length of time unless they will be used as raw materials for processing. For some vegetables the time elapsed from harvesting to consumption or processing is the only time spent in storage. On the other hand, vegetables such as potato, yam, sweet potato, garlic, and ginger can be kept *in situ* for several months after they attain maturity and removed from storage environments before the rainy season to prevent rotting and sprouting. *In situ* storage of these vegetables is easy and economical due to limited expenditure and fabrication for storing. Underground storage in pits and trenches by mounting soil on the surface is most suitable for short-term storage. Hay or straw and then soil are used to protect the surface from water leakage and freezing. Pits are used for storing beet, potato, carrot, turnip, cabbage, parsnip, etc. The disadvantages of underground storage include expensive labor, variable climatic conditions, and adverse weather [18, 19].

Refrigerated or cold storage has established itself as the most accepted storage method all over the world. Its use is determined by the cost and benefit considerations. The storage life of selected fruits and vegetables at given temperature and humidity is given in Table 5.5. In general, commodities store well above the chilling injury threshold temperatures for tropical and subtropical fruits and above the freezing point for temperate fruits. Comprehensive compilation of storage information in terms of optimum temperature, and humidity in normal and modified or controlled atmospheres can be found in several references [2, 7, 9–11, 13, 17–19]. The Optimal Fresh Database, developed by the Sydney Postharvest Lab and Food Science Australia [20], presents information on refrigerated container/cold room recommendations, produce properties (freezing point, humidity, storage time at ambient and at optimal temperature), suitability and conditions for controlled atmosphere storage, respiration and heat transfer, compatibility in mixed storage, and seasonal availability for many fruits and vegetables.

Rooms used for cold storage should provide adequate insulation, working space, vapor barrier, and doors that disallow the ingress of air from outside. Good air circulation is required to assure uniform temperature and humidity in the room. A minimum clearance of 100 mm should be kept from the walls and floors and between pallets while stacking the produce [21]. An efficient operation of storage system involves: acceptance of only high quality produce for storage; rapid cooling of the produce to the storage temperature; storing only compatible mixed load; maintenance of recommended temperature, humidity, and/or gaseous atmospheres; precise monitoring and control of temperature, humidity; and efficient product movement to avoid excessive storage [22].

Often it is necessary to store or transport several types of produce at once. However, the produce should be compatible with one another. The factors that determine the compatibility of products are (i) temperature, (ii) relative humidity, (iii) production of ethylene, (iv) sensitivity to ethylene, (v) production and absorption of objectionable odors or flavors, and (vi) difficulties in loading shipping containers or stores of different

TABLE 5.5
Optimum Storage Temperature and Humidity Conditions for Fresh Fruit and Vegetables

Produce	Optimum Temperature, ˚C	Freezing Temperature	Relative Humidity, %	Storage Life
Apple	–1 to 4.4	–1.7	90–95	1–12 months
Asparagus	2.2	–1.1	95–100	2–3 weeks
Blueberries	0.6–1	–2.2	90–95	2–3 weeks
Broccoli	0	–0.6	95–100	2 weeks
Cabbage	0	–1.1	95	2–3 months
Cucumbers	7.2–10	–0.6	95	2 weeks
Eggplant	7.8–12.2	–0.6	90–95	1 week
Green beans and field peas	2.8–7.2	–0.6	95	5–10 days
Leafy vegetables	0	–1.1	95	1–2 weeks
Onions	0	–0.6	70	2–3 months 6–8 months in CA
Peaches	0	–0.6	95–98	2–4 weeks
Peppers	7.2–10	–0.6	90–95	2–3 weeks
Potatoes	3.3–4.4	–0.6	90–95	5–8 months
Strawberry	0	–0.6	90–95	5–7 days
Sweetcorn	0	–0.6	90–98	5–7 days
Sweet potatoes	12.8	–0.6	90	6–12 months
Tomatoes, pink	8.9–10		85–95	7–14 days
Turnips	0	–1.1	95	4–5 months
Watermelon	10–15.6	–0.6	90	2–3 weeks

Source: Wilson et al. [15].

sizes and shapes. Compatibility groups for the storage of fruits and vegetables are given in Table 5.2.

5.3 POSTHARVEST TREATMENTS

Postharvest treatments generally aim at preserving and/or enhancing the quality of fruits and vegetables by controlling the physiological, mechanical, and pathological agents responsible for both postharvest losses and degradation of quality. These may be described as physical and chemical treatments.

5.3.1 Physical Treatments

Physical treatments are mainly considered to be chemical-free, such as cleaning, washing, waxing, heat, and irradiation, and result in an absence of chemical residue in the products. They involve less hazard as compared to the chemicals.

5.3.1.1 Cleaning and Washing

The main goals of cleaning are to (i) eliminate surface dirt and soil particles and contaminants, (ii) remove residues of pesticides, fertilizers, and chemicals used during production, (iii) reduce the microbial load, and (iv) enhance the appearance of the produce. Cleaning can be accomplished using air (dry) or water (washing). Dry brushing with or without air blast may be used to remove loose scales, soil, or dust in products such as onions, garlic, potato, sweet potato, cantaloupes, and melons.

The effectiveness of the washing depends on: the amount of water used, characteristics of water (acidity, hardness, mineral content, temperature, and the initial level of contamination), force applied, use of brushing and rubbing aids, etc. The water used for cleaning should be of acceptable quality and must be filtered and sanitized before reuse. Washing is not an effective method for removing fungi from the infected tissues and may even predispose produce to decay organisms and deplete the protective wax layer. Washing may also lead to water-soaked appearance and moisture penetration, which may aid in pathogen access through the wounds. This is the reason why strawberries, mushrooms, cucumber, and cherries are not generally washed. If fruit is excessively dirty a detergent may be used prior to a sanitizing treatment. The final rinse should be carried out using clean water. Removal of excess surface water by blotting rollers or blowing air over fruits may be necessary to avoid infection and subsequent decay in stone fruits and potatoes [3].

5.3.1.2 Coating and Waxing

The presence of surface wax is a natural defense mechanism in fruits and vegetables against water loss and invasion from pests and disease-causing organisms. Rough handling, approaching senescence, and washing deplete natural waxes. Surface coating using wax or hydrophobic substances has been used since ancient times to improve the appeal and acceptability for the consumer, ease of packing and handling, and to extend the shelf life by reducing weight (water) loss. Retention of color, firmness, flavor, and the prevention of loss of weight result from (i) reduction in the rates of respiration and transpiration, (ii) protection from insects, pests, and fungi causing diseases and deterioration, (iii) generation of local modified atmosphere, (iv) protection from mechanical injuries, and (v) curing of tiny injuries and scratches on the surface [3]. Significant economic benefits accrue by waxing due to resultant water loss reduction to an extent of 30–50% in normal commercial handling and storage conditions [22]. However, coating may not be always favorable as modification of the internal atmosphere can reduce the available oxygen leading to fermentation, which can be precluded by only a thin layer of wax to allow gas exchange through it. The literature related to coatings has been reviewed by Baldwin [23].

Commercial formulations used in coating consist of long-chain fatty alcohols, synthetic resins, chitosans, and other sugar derivatives as active coating agents, and substances to assist in coating, e.g. emulsifying and wetting agents. Commonly used waxes are carnauba, shellac, candelilla, beeswax, paraffin wax, and vegetable oils. Waxing formulations can be used as carriers of chemicals for preventing fungal infestation, senescence, and other physiological disorders. Coating formulations are applied by spraying, fogging, or brushing onto the produce followed by drying using cold or hot air. Examples of fruits and vegetables normally waxed are apples, pears, banana, citrus fruits, cucumber, pepper, and tomato.

5.3.1.3 Heat Treatment

Moderate heating has been used since ancient times as a quarantine measure to control insect pests and pathogens, and to increase the shelf life of plant produce. Deregistration of chemicals used to control physiological disorders, insect pests, and pathogens and consumer demand for produce with no chemical exposures have fueled increased interest in the use of heat in postharvest management of quality. Heat treatment has a positive effect on maintaining fruit quality by preventing and/or controlling incipient fungal and insect infestation, reducing the rate of ripening, increasing sweetness and reducing acidity in fruits, and in reducing impact of storage disorders, such as superficial scald and chilling injury [24–27]. The heating schedules have been specified in terms of temperature and time of heating for each commodity and the purpose for which the treatment is used. Tables 5.6, 5.7, and 5.8 list the conditions of heat treatment used for insect disinfestations, disinfection, and control of physiological disorders and enhancing quality, respectively. The effectiveness of the treatment depends on the nature of the produce and its sensitivity to heat, temperature and time of heating, the heating method used, and any supplemental treatments such as combinations with antioxidants or controlled atmospheres. Exposing the produce to a temperature conditioning treatment before storage by incubating the produce at ambient temperature for a certain length of time may also be beneficial.

Hot air, vapor, and hot water can be used as sources of heat. Hot water treatment has the advantages of low cost and relatively simple application equipment. The vapor heat treatment is relatively expensive due to costs associated with the equipment and process operation. It requires an airtight and moisture-proof treating room equipped with temperature and humidity controls and a boiler for steam generation. In general,

TABLE 5.6
Typical Heat Treatments for Controlling Insects in Selected Fruits and Vegetables

Commodity	Insect	Temperature ˚C/Time	Heating Medium Used
Apples	Codling moth (*Cydia pomonella*)	44/120 mins followed by 0/4 weeks	Hot air or vapor
	Leafroller (*Cnephasia jactatana*)	40/10 h and 45/5 h in reduced O_2	Hot air and CA
	Light brown apple moth (*Epiphyas postvittana*)	40/17–20 h in reduced O_2 and	Hot air and CA
	Obscure mealy bug (*Pseudococcus*	slightly elevated CO_2	Hot air and CA
	longispinus)	40/10 h and 45/5 h in reduced O_2	Hot water and ethanol
	Two spotted spider mite (*Tetranychus urticae*)	45/13 min in 50% ethanol	
Avocado	Mediterranean fruit fly (*Ceratitis capitata*)	40/24 h	Hot air
	Melon fruit fly (*Dacus cucurbitae*)	40/24 h	Hot air
	Queensland fruit fly (*Bactrocera tyroni*)	46/3 min followed by 1/7 days	Hot water and benomyl
Citrus fruits	Mexican fruit fly (*Anastrepha ludens*)	44/2 h with CA	Hot air and 1% O_2
	Caribbean fruit fly (*Anastrepha suspense*)	51.5/125 min	Hot air
	Fuller's rose beetle (*Asynonychus gomani*)	52/8 min	Hot water
Mango	Mediterranean fruit fly (*Ceratitis capitata*)	47/15 min	Vapor heat
	Caribbean fruit fly (*Anastrepha suspense*)	51.5/125 min	Hot air
	Papaya fruit fly (*Bactrocera payapae*)	47/15 min	Vapor heat
	Queensland fruit fly (*Bactrocera tyroni*)	46.5/10 min	Vapor heat
Pear	Codling moth (*Cydia pomonella*)	44/120 mins followed by 0/4 weeks	Hot air and vapor
	Light brown apple moth (*Epiphyas postvittana*)	30/30 h in reduced O_2	Hot air and CA
	Oriental fruit moth (*Grapholita molesta*)	30/30 h in reduced O_2	Hot air and CA

Sources: Lurie and Klein [24]; Lurie [27].

both hot water and vapor heat treatment can cause excessive tissue damage and peel injury compared to forced hot air.

Since surface injuries are sites for infection by decay organisms, heating the surface of fruits to a few degrees below the tissue injury threshold eradicates or delays the development of incipient infections by pathogenic fungi [3]. Typical heat treatments used for controlling pathogens of selected fruits and vegetables are shown in Table 5.7. Curing is a postharvest healing process of the outer tissues of root crops by the development of a wound periderm by the

TABLE 5.7
Typical Heat Treatments for Controlling Pathogens

Commodity	Pathogen	Temperature ˚C/Time	Heating Medium Used
Apples	Grey mold (*Botrytis cinerea*)	38/4 days	Hot air with $CaCl_2$ dip
	Blue mold (*Penicillium expansum*)	38/4 days	Hot air alone or combination with $CaCl_2$ dip
Banana	Crown rot (*Chalara paradoxa*)	45/20 min or 50/10 min	Hot water
Grapefruit	Green mold (*Penicillium digitatum*)	46/6 h or 59–62/15 sec	Hot water
Lemon	Green mold (*Penicillium digitatum*)	45/2.5 min	Hot water with 2% sodium carbonate
		36/3 days	Hot air
Mango	Black spot (*Alternaria alternata*)	60–70/15–20 secs	Hot water
	Antracnose (*Colletotrichum gloeosporrioides*)	46–48/24 sec to 8 min	Hot water, vapor
	Stem end rot (*Diplodia natalensis*)	51.5/2h mins	Air
		51.5/2h mins	Hot air and water
Orange	Green mold (*Penicillium digitatum*)	41–43/1–2 min	Hot water and 6% sodium carbonate
		53/3 min	Hot water
Papaya	Stem and surface rots (*Botryodiplodia theobromae*)	49/20 min or	Hot air
	Stem and surface rots (*Mycospharella* spp.)	32/33 min first and then 49/20 mins	
Cactus pear	Blue mold (*Penicillium italicum)*	38/24 h or 55/5 min	Hot water or air
Pepper	Grey mold (*Botrytis cinerea*)	50/3 min	Hot water
Strawberry	Grey mold (*Botrytis cinerea*)	45/15 min	Hot water
Tomatoes	*Rhizopus stolonifer*	50/2 min	Hot water

Source: Lurie and Klein [24].

TABLE 5.8
Optimum Conditions for Curing Vegetables

Commodities	Temperature, ˚C	Relative Humidity (%)	Days
Cassava	30–40	90–95	2.5
Cassava	25–40	80–85	7–14
Potato	15–20	90–95	5–10
Sweet potato	30–32	85–95	4–7
Sweet potato	29–32	80–90	4–7
Sweet potato	30–33	85–95	5–7
Yams	32–40	90–100	1–4

Sources: Ravi et al. [29]; Kitinoja and Kader [1].

application of heat. The periderm acts as an effective barrier against infection and water loss. The purposes of curing are (i) to heal wounds of tubers and bulbs sustained during harvesting, (ii) to strengthen the skin, (iii) to dry superficial leaves, such as onion bulbs, in order to prevent microbial infection during storage and distribution, (iv) to develop desired skin color (onion), (v) to reduce water loss during storage in potatoes, sweet potatoes, cassavas, yams, onions, and garlic [30]. Curing is carried out at the farm level by subjecting produce to high temperatures and humidity for a given duration. If local weather conditions permit, crops can be undercut in the field, windrowed, and left to dry for 5 to 10 days. The dried tops of the plants can be arranged to cover and shade the bulbs during the curing process, protecting the produce from excess heat and sunburn [1]. The optimum curing conditions for different crops are given in Table 5.8. One day or less at 35 to 45°C and 60 to 75% relative humidity is recommended if forced heated air is used for curing onions and other bulbs [1].

Heat treatment can also assist in controlling the postharvest disorders and enhancing the shelf life of fruits and vegetables by the formation of areas of amorphous wax and fewer surface cracks in apples after heat treatment (Table 5.9). Heating apples to 38°C for 3 or 4 days before storage suppressed softening [31], and decreased storage disorders such as superficial scald and bitter pit [32]. Pre-storage heating plus calcium dip has shown a synergistic effect in maintaining fruit firmness [25] and decreasing storage disorders. Prolonged exposure to elevated temperatures must be avoided to reduce weight loss and loss of ripening ability. Heating at 38°C for various holding times has been found to be effective in preventing chilling injury for produce stored at 2˚C for 4 weeks.

TABLE 5.9
Typical Heat Treatments for Controlling Physiological Disorders and Enhancing Quality of Selected Fruits and Vegetables

Commodity	Physiological Disorders/ Injury	Temperature ˚C/Time	Heating Medium Used
Apples	Scald and improving firmness	38/4 days or 42/2 days	Hot air
Asparagus	Inhibition of curvature	47.5/2–5 min	Hot water
Avocado	Browning Pitting	38/3–10 h followed by 40/30 min 38/60 min	Hot air and water Hot water
Cactus pear	Rind pitting	38/24 h or 55/5 min	Hot water or air
Citrus fruits	Rind pitting	34–36/48–72 h 50–54/3 min	Hot air Hot water
Guava	Increased hardness and yellowing	46/35 min	Hot water
Green pepper	Pitting	40/20 h	Hot air
Mango	Pitting	38/2 days or 54/20 min	Hot air
Tomatoes	Pitting	38/2–3 days 48/2 min or 42/1 h	Hot air Hot water

Source: Lurie and Klein [24].

5.3.1.4 Irradiation

Irradiating fruit and vegetables with ionization energy as X-rays, gamma, or electron beam has been investigated to inhibit sprouting in tubers (potato) and bulbs (onion), to delay ripening and senescence in tropical fruits such as mango and papaya, to control infestation by insects such as fruit fly and seed weevils in mangoes, to pasteurize fruit surfaces, and to improve microbial properties of fruits and to improve process efficiency. However, the use of irradiation is limited by people's perception of radiation, the cost of the treatment process, and phytotoxic effects induced in the treated produce that adversely affect quality. Irradiation is very effective in controlling insect disinfestations as a quarantine measure and for sprout inhibition. Table 5.10 lists the principal uses and the doses of irradiation for fruit and vegetable products [33, 35]. Only low- to medium-dose irradiation (0.05 to 3 kGy) is commonly employed. Irradiation is

TABLE 5.10
Application of Irradiation for Fruit and Vegetables

Purpose	Products Subjected to	Dose (kGy)
Sprout inhibition	Potatoes, onion, garlic, ginger, yam	0.05–0.15
Insect disinfestations	Fresh and dried fruits	0.15–0.5
Delaying maturity and senescence	Fresh fruit and vegetables	0.25–1.0
Extending shelf life	Strawberries, mushrooms	1.0–3.0
Improving technological properties	Grapes (juice recovery), dehydrated vegetables (reduced cooking time)	2.0–7.0

Source: Anon [33].

gaining popularity as an alternative to chemical fumigation and is now accepted and approved by about 60 countries as a quarantine treatment [33]. It has been approved by FDA of the US for sprout inhibition of white potatoes, ripening delay in fruits, disinfestations of fruit and vegetables, and as a quarantine measure for mangoes and papaya for the control of fruit fly and stone weevil [33, 35]. Normal doses required for insect disinfestations have been found to produce no phytotoxicity in apple, cantaloupe, cherry, currants, date, guava, honeydew melon, kiwi, litchi, mango, muskmelon, nectarine, papaya, peach, prune, raspberry, strawberry, and tomato (ICGFI task report 13, 1991). Currently, 26 fruits and vegetables are allowed in Australia to be irradiated with a minimum dose of no lower than 150 Gy and no greater than 1 kGy (Food Standards Australia New Zealand Code Standard 1.5.3). The code also specifies mandatory labeling of all irradiated foods.

5.3.2 Chemical Treatments

Chemicals have been used in fruits and vegetables to control microorganisms causing decay and diseases, infestations due to pests, physiological disorders, and to enhance the quality of produce. Chemicals can be applied as spraying and dipping solutions or emulsions, electrostatic spray, dusting, fumigation, thermal fogging, and as adsorbent pads. The choice of a particular chemical treatment and the dose depends on intended use, phytotoxicity, residue, and degradation. These compounds should be selected for both efficacy and minimal interference with the natural color, flavor, and odor of the product along with toxicity, residue in the product, and legal aspects before applying any chemical treatment.

5.3.2.1 Disinfestation and Decay Control

Fruits and vegetables are exposed to deterioration due to insect pests, fungi, and bacteria at any time from production until consumption or processing. Insect pests cause significant losses worldwide [3, 7, 19]. Similarly, pathogens significant to fruits and vegetables have been covered in the literature [19, 22, 28]. The list of chemicals approved for use as insecticides, fungicides, or bactericides is shrinking due to their environmental impact and toxicity. The general trend is to observe strict hygiene in the production and handling of the produce, to use physical methods (heat and modified atmospheres), and to use chemicals that are generally regarded as safe (GRAS) or biological agents.

Chemicals are applied as insecticidal dips or fumigants for postharvest disinfestation of fruits and vegetables. Gaseous sterilants used for quarantine treatments include ethylene dibromide, methyl bromide, acrylonitrile, carbon disulfide, carbon tetrachloride, ethylene dioxide, hydrogen cyanide, phosphine, and sulfuryl fluoride. The use of methyl bromide has been now phased out under the Montreal Protocol and may be permitted only when the importing countries specify it as a quarantine measure for importation (www.postharvest.net.au/postharvest-fundamentals/quarantine-treatments/quarantine-treatments/).

Incipient growth of both fungi and bacteria in fruit and vegetables used as raw material for processed products causes defects in sensory (color, texture, and flavor) and microbiological quality of end product. Increased softening upon canning, pickling, and acidic or alcoholic flavor in fruit juices are some examples of spoilage symptoms. Fungicides are applied to fruits and vegetables both as pre- and/or postharvest treatments depending on the nature of produce, the target pathogen, market life, and cost. Local and international laws strictly control the use of fungicides. Table 5.11 lists chemicals used in the postharvest control of common pathogens. The chemicals that are most effective in controlling fungi are thiabendazole, dichloran, imazalil, sulfur, and its derivatives. However, increased resistance to these fungicides is a problem.

Sulfur and its derivatives are effective in controlling fungi and molds in fruits in the form of fumigation, dipping, and spraying or pads. Fumigation of grapes with sulfur dioxide is standard practice for controlling decay. Salunkhe et al. [3] fumigated grapes with 1% sulfur dioxide (v/v) for 20 min immediately after harvest to sterilize the surface of the berries and any injuries made during harvest. The initial treatment may be followed by periodic fumigation with 0.25% sulfur dioxide at 7- to 10-day intervals during storage. In some cases, the color and texture of fruits are also improved by sulfur dioxide treatment. Two major disadvantages of sulfur dioxide use are corrosion to metal surfaces of the storage and treatment chamber and bleaching the point of attachment of the stem to the berry [3]. Bisulfites can be used as pads in cartons carrying grapes to control molds [1].

Nitrogen trichloride (NCl_3) fumigation treatment has been used extensively to control the sporulation and spread of pathogenic fungi during storage. It is hydrolyzed in a

TABLE 5.11
Chemicals Used in Postharvest Control of Pathogens in Fruits and Vegetables

Chemical	Pathogen Controlled	Host
Inorganic sulfur as SO_2 gas or salts	Monillia, Botrytis	Grapes
Organic sulfur compounds (e.g. thiram)	*Alternaria*	Strawberry, banana
Phenols (sodium o-phenylphenate)	*Penicillium*, bacteria, and fungi	Citrus fruits
Triazoles (imazalil)	*Penicillium, Alternaria*	Citrus fruits
Hydrocarbons (Biphenyl)	*Penicillium, Diplodia*	Citrus fruits
Organic acids (dehydroascorbic acid, sorbic acid, acetic acid, formic acid)	Botrytis and other fungi	Strawberry
Benzimidaxoles (benomyl)	*Penicillium, Collectotrichum, Sclerotinia*, Botrytis	Stone fruits, carrots

Sources: Thompson [19]; Wills and Golding [22].

moist environment to HOCl, which is probably responsible for decay control and for corrosion [3]. In recent years, its use has declined because of its corrosion problems. Biphenyl may be used after impregnation into fruit wraps or onto the paper sheets placed at the bottom and the top of the fruit container to inhibit fungi. It sublimes into the atmosphere surrounding the fruit and inhibits the development of decay. The main problem with using biphenyl is that it leaves a residue on the surface, which gives off a slight hydrocarbon odor [3].

There is a trend to use essential oils and natural substances as fungicides which have low mammalian toxicity. *Trans*-cinnamaldehyde is more effective as an antifungal agent when applied as an aqueous solution than when in the gas phase, since it oxidizes to cinnamic acid when exposed to air [36]. The surface treatment of tomatoes with *trans*-cinnamaldehyde is effective in reducing the number of potential spoilage bacteria and fungi [37] before storage under modified atmosphere. Similarly, Ryu and Holt [38] demonstrated the effectiveness of an aqueous solution of cinnamon oil for the surface disinfection of apples. The effectiveness of treatment can be improved by the addition of Tween 80 (0.05%) and ethanol (3%), which assist in dissolving the wax layer, to apples near fungal spoilage.

In addition to following strict hygiene through good agricultural practices at the farm level and hazard analysis and critical control point (HACCP) protocols through the distribution channel, the potential alternatives of chemical treatments for control of diseases and pests are (i) low and high temperatures treatments (Table 5.7), (ii) atmospheres with very low oxygen and/or very high carbon dioxide, and (iii) atmospheres with natural insecticidal volatiles, and (iv) irradiation (Table 5.10), (v) using radiofrequency for control of insects [39, 40], and (vi) using biological control for wound-invading necrotrophic pathogens [2, 19, 41, 42]. Certain bacteria, e.g. *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Geobacillus stearothermophilus*, are effective in controlling diseases such as anthracnose and stem end rot of mangoes, and *Candida guilliermondii* for molds (*Penicillium* spp. in citrus fruits). These biological agents are very useful in combination with chemicals for disease control [19].

Atmospheres with very low oxygen (≤0.5%), and/or very high carbon dioxide (≥50%) are insecticidal. Insecticidal atmospheres can be used for mango, papaya, and avocado as a quarantine measure. However, not all fresh fruits and vegetables can tolerate such extreme atmospheres. The advantages of using insecticidal atmospheres include (i) absence of toxic residue on the produce, (ii) environmentally safe, and (iii) competitive in cost with chemical fumigants. The disadvantages, however, are that it takes longer to kill insects with insecticidal atmospheres than with fumigants, and may cause anaerobiosis and fermentation in fresh horticultural crops [43].

Water disinfection is required to prevent the introduction and spread of postharvest diseases and food-borne infections of human pathogens. The common disinfectants and sanitizers used and their mechanisms of action are given in Table 5.12. Chlorine compounds are used to sanitize water used in cleaning raw produce, fresh and cut fruit and vegetables, and food processing equipment. Chlorine is very reactive and the most acceptable disinfectant due to its antimicrobial activity against bacterial cells and spores, reduction in the formation of biofilms on the surface of handling equipment, and low residual effect. The use of chlorinated water at 10–200 mg/kg rapidly kills vegetative cells of yeast and bacteria. The recommended levels of chlorine in wash water are 1–3 ppm for rinsing and 50 ppm for sanitizing [6]. Chlorine is used as a gas or sodium or calcium hypochlorite salts. When added to water the following reactions take place:

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

$$NaOCl + H_2O \rightarrow HOCl + NaOH$$

$$Ca(OCl)_2 + H_2O \rightarrow HOCl + Ca(OH)_2$$

$$HOCl \Leftrightarrow OCl^- + H^+$$

The disinfectant activity of chlorine compounds depends on several factors, which include the form of the chlorine, pH,

TABLE 5.12
Sanitizers Used in Disinfection of Wash Water

Sanitizer	Activity	Oxidation Capacity (eV)	Concentration	Effectiveness
Peracetic acid	Oxidant	1.81	Up to 80 ppm	pH 1–8, sensitive to organic matter
Hypochlorites	Oxidant	1.36 (for sodium hypochlorite)	1–3 ppm for rinsing 50 ppm for sanitizing	pH 6–7, sensitive to organic matter
Chlorine dioxide	Oxidant	1.57	Up to 5 ppm	pH 6–10, less sensitive to organic matter in comparison to hypochlorites
Hydrogen peroxide	Oxidant		0.5%	Sensitive to organic matter
Ozone	Oxidant	2.07	2 ppm	pH 6–8, sensitive to organic matter, breaks down to O_2 rapidly, corrosive to equipment
UV light	Disruption of genetic material	-	40,000 μw-sec/cm^2	Independent of pH sensitive to organic matter
Iodophore	Oxidant		6–13 ppm of free iodine	pH 2–5, sensitive to organic matter, corrosive

temperature, contact time, and presence of organic matter. Of many forms of chlorine, hypochlorous acid (HOCl) is the most effective as a disinfectant. The pH of the water should be maintained at 6–6.5 to ensure optimum disinfectant activity and to avoid the formation of gaseous chlorine, which causes irritation to workers at <6 pH. It is necessary to maintain the effective concentration of the acid in the wash water especially when water is recycled. In commercial operations, 50 to 100 ppm chlorine at pH 7.5 to 8.5 is frequently employed when the washing water carries a substantial amount of soil and organic matter. Sulfamic acid and other amines can be added to water to form *N*-chloramines which tend to stabilize the concentration of active chlorine. Sodium *o*-phenylphenate (SOPP) is also used occasionally to reduce the number of pathogenic microorganisms in produce treatment water. SOPP is non-corrosive and improves the stability of the solution, and compatibility with chemicals, which react with chlorine. Further stabilization of chlorine solution is possible by adding 2-aminobutane (phosphate) in addition to SOPP [3].

Alternatives to chlorine as a disinfectant are ultraviolet light (UV), ozone, and organic acid formulations such as peracetic acid. The UV light disrupts the DNA and can be used for sanitation of water and surfaces. Most disinfectants are strong oxidizing agents, and their disinfectant power is related to their oxidation capacity. Based on this capacity, ozone is a very effective disinfectant; however, it degrades rapidly to oxygen and loses its activity. Besides wash water, disinfection of storerooms can also be done by spraying with 5% Lysol or 2% formalin, painting of walls with antifungal chemicals, and fumigation with paraformaldehyde [44].

Accurate monitoring, control, and recording of all the factors influencing disinfection are key elements of a sound disinfection program. Oxidation-reduction potential (ORP) is widely accepted as a key indicator of water disinfection potential for real-time monitoring and recording of the disinfection process in postharvest systems. Maintaining an ORP value of 650–700 mV for a few seconds can inactivate most spoilage and food-borne bacteria such as *Escherichia coli* and *Salmonella* spp. [45].

5.3.2.2 Ethylene Removal

Ethylene, a plant hormone, affects the physiological processes of ripening and senescence, which signal cell death. Exposure to ethylene (1 ppm) can reduce the postharvest life of many fruits and vegetables by hastening the onset and increasing the rate of senescence, softening, and loss of green color. Damaged or diseased fruits produce more ethylene and have a catalytic effect in stimulating: softening of tissues, discoloration, bitterness due to production of isocoumarins in carrots, russet spotting in lettuce, browning of tissues in vegetables such as eggplant and sweet potatoes, sprouting of potatoes, development of woodiness in asparagus, shattering of berries, such as blackberries and raspberries, loss of green color in vegetables, and stimulation of growth of fungi (*Penicillium italicum* in oranges, *Botrytis cineria* on strawberries).

The ethylene control strategy includes the prevention of exposure of plants to biologically active levels of ethylene, reducing the perception of atmospheric ethylene, and preventing the tissue response to perceived ethylene [46, 49]. Ethylene damage can be reduced by (i) adequate ventilation, (ii) reduction of O_2 and increase in CO_2 levels, (iii) reducing temperature, (iv) avoiding storage and transportation of ethylene producers and sensitive produce, and (v) reduction of ethylene by forcing air through filters of activated charcoal (brominated), treatment with silver thiosulphate, potassium permanganate ($KMnO_4$) or purafil, 1-methyl cyclopropene (MCP) or EthylBlock, and oxidation by UV light. Potassium permanganate, the most accepted ethylene remover used commercially, oxidizes ethylene into ethylene glycol and is often incorporated into different carrier materials such as activated alumina, silica gel etc. It is applied as sachets, tubes, and blankets in storage and transportation of fresh fruits and vegetables. When used in conjunction with modified atmosphere packaging, the use of $KMnO_4$ increases the shelf life of banana to 21 days from 7 days in air [22]. MCP helps in delaying the rise in respiration and preventing tissue softening and incidence of physiological disorders such as superficial scald in apples. The response of fruit to MCP depends on the type of fruit, cultivar, maturity, the application method, and exposure levels of MCP used [13, 47–49]. MCP has been approved at concentrations up to 1 ppm for use on apples, apricots, avocados, kiwifruit, mangoes, nectarines, papayas, peaches, pears, persimmon, plums, and tomatoes in the United States [13].

5.3.2.3 Controlled Ripening and Color Development

Climacteric fruits, such as banana and mangoes, are harvested well before they are fully ripe to avoid mechanical injury and are ripened during storage or transport under controlled conditions of temperature, relative humidity, and ethylene gas just before consumption or processing. Controlled ripening facilitates uniform development of color, texture, and flavor.

Ethylene is the most active ripening agent. Ethylene is a product of incomplete combustion of fuels such as charcoal and is highly flammable when pure, and hence it is used in relatively low concentrations (<3%). It can be generated by passing ethyl alcohol over a bed of activated column. Ethephon (2-chloroethyl phosphoric acid) may be used as a source of ethylene for ripening of fruits. The amount of ethylene released depends on the fruit pH and relative humidity. Acetylene, generated by mixing water with calcium carbide salt, can also be used as a ripening agent; however, it is 100 times less effective compared to ethylene. Endogenous or exogenous ethylene is used for controlled ripening of fruits (banana, mangoes) and development of a uniform color of the produce (tomatoes and citrus fruits) under controlled conditions. The ripening effect depends on the concentration of ethylene, exposure time, relative humidity, and respiratory behavior of fruits. A batch process for ripening bananas consists of exposing fruits to ethylene concentrations of 20–200 µl/L in a sealed chamber for 24 hours followed by ventilation to avoid buildup of ethylene and carbon dioxide before removing fruits. The chamber temperature is maintained at 15–21°C by controlling the airflow in a forced-air system. Initially, RH

TABLE 5.13
Typical Conditions for Postharvest Ripening and Color Development of Fruits

Fruit	Ethylene Concentration (ppm)	Temperature (°C)	Time (hr)	Application
Avocado	10–100	15–18	12–48	Ripening
Banana	100–150	15–18	24	Ripening
Honeydew melon	100–150	20–25	18–24	Ripening
Kiwi fruit	10–100	0–20	12–24	Ripening
Mango	100–150	20–22	12–24	Ripening
Orange	1–10	20–22	24–72	Degreening
Tomato	100–150	20–25	24–48	Color development

Source: Adapted from Kitinoja and Kader [1].

is maintained first at 85–90% level to preclude water loss, development of blemishes, and poor color formation, and then reduced to 70–75% to avoid skin spotting at later stages of maturity [22]. Table 5.13 lists typical ripening conditions used for some fruits using ethylene as a main ripening agent [1].

5.3.2.4 Delaying Ripening, Senescence, and Sprouting

Ripening is undesired in most vegetables, except in the case of tomatoes, and signals the onset of senescence in fruits. Various plant growth regulators can be used at various stages of production and postharvest handling for delaying ripening, color degradation, and sprouting [3]. These chemicals can be applied as a dip or spray. Table 5.14 lists the chemicals used, their effect, and products for which these are applied.

5.3.2.5 Treatment with Divalent Cations

Calcium and other divalent ions are useful in delaying senescence and maintaining the quality of fruits and vegetables by altering respiration, protein and chlorophyll content, and membrane fluidity [2]. Calcium application also reduces

TABLE 5.14
Chemicals Used for Delaying Ripening, Senescence, and Sprouting

Chemical	Effect	Produce used
Cytokinin	Delays chlorophyll degradation and senescence	Leafy vegetables (spinach), pepper, bean, cucumber
Benzyladenine	Delays chlorophyll degradation and senescence	Cherry
Benzylaminopurine	Delays chlorophyll degradation and senescence	Sweet cherry, cauliflower, endive, parsley, snap beans, lettuce, radish, onions, cabbage, Brussels sprouts, broccoli, mustard greens, radish tops, celery, asparagus
Kinetin	Delays chlorophyll degradation and senescence	Leafy vegetables (spinach), pepper, bean, cucumber
Gibberellin	Retards maturation, ripening, and senescence, delays chlorophyll degradation, increases peel firmness, delays accumulation of carotenoids	Tomato, banana, kiwi fruit, citrus fruits (orange, grapefruit)
Maleic hydrazide and its analogues	Sprout inhibition	Onion, sugar beet, turnip, carrot, potato
	Delays ripening	Mango, tomato, sapota fruit
Alar	Delays deterioration and discoloration	Mushroom
	Preservation of chlorophyll	Leaves of beans
	Inhibition of synthesis of solanine	Potato
Cytocel	Retards senescence and deterioration	Vegetables
IPC	Control sprouting	Root crops
CIPC	Control sprouting	Potato
Tecnazene	Control sprouting and fungi	Root crops
Calcium	Delays chlorophyll degradation and senescence	Vegetables
1-MCP	Delays ripening	Apple, banana, pear, tomato, peach, apricot, nectarine, plum, mango, avocado

Sources: Salunkhe et al. [3]; Valero et al. [47].

TABLE 5.15
Diseases and Disorders Controlled or Prevented by Calcium and Other Divalent Ions

Disease/Disorder	Produce
Blossom end rot	Tomato, pepper
Tip burn	Lettuce
Internal browning	Potatoes
Bacterial and fungal decay	Potatoes, carrots
Bitter pit	Apples
Incidence of molds	Cucumber
Chlorosis	Most vegetables, potatoes

texture loss, ethylene production, browning, development of bitterness, and microbial decay [2, 50, 51] and increases the concentration of ascorbic acid.

Low calcium levels are strongly related to high incidences of bitter pit in apples, blossom end rot in tomatoes, tip burn in lettuce, and hollow heart and brown center in potatoes [6, 50]. Table 5.15 lists the diseases and disorders associated with calcium and other divalent ions. Chlorosis, loss of green color, is induced by a deficiency of magnesium. Since soil fertilization with calcium salts is not effective in raising the level of calcium in fruits, orchard sprays or postharvest dips or vacuum and pressure infiltrations are used to increase the levels of calcium in tissues.

Calcium exists as calcium pectate in the middle lamella of the cell wall cementing the structure of the plant cell. The loss of calcium from calcium pectate leads to the softening of fruits. The addition of calcium improves texture by reacting with pectic acid to form calcium pectate. Sprays and dips of calcium chloride solutions delay softening and senescence of fruits by the formation of cross-links between polygalacturonide chains and calcium in cell walls, thus resulting in an extension of shelf life. Calcium is not used in other fruits except in the case of apples to avoid skin injury due to higher calcium uptake and rot development [22].

5.3.2.6 Treatment with Antioxidants

Superficial scald may develop due to oxidation of α-farnesene during cold storage of apples, as natural antioxidants are lost. This disorder can be controlled by the application of antioxidants. Diphenylamine (0.1–0.25%) or ethoxyquin (6-ethoxy-2, 2,4-thimethyl-1,2-dihydroquinoline) (0.2–0.5%) are two antioxidants used commercially. These may be used as spray or dipping solutions or can be added to the formulation used for the waxing of fruits.

5.4 CONCLUSION

Fruits and vegetables undergo several postharvest handling operations before reaching the consumer in fresh form or a processor as a raw material for further processing. An overview of postharvest handling operations and treatments has been provided in this chapter as this knowledge is essential to extend the shelf life and to maintain the quality of these perishable products.

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6 Structure, Composition, and Harvesting of Grains and Pulses

Ajit K. Mahapatra and Yubin Lan

CONTENTS

6.1 STRUCTURE OF CEREAL GRAINS AND LEGUMES

6.1.1 Cereal Grains

Cereals are often considered among the first cultivated crops. They are herbaceous plants belonging to the grass family Graminaceae (the only exception being buckwheat) [1]. Cereal grains such as wheat, rice, corn, barley, oat, rye, sorghum, and millet are used primarily for human consumption and animal feed. These are also used in the manufacture of beverages and industrial products (adhesives, starch). Cereal crops are energy-dense, containing 10,000–15,000 kJ/kg, about 10–20 times more energy than most succulent fruits and vegetables [2]. Nutritionally, they are important sources of dietary protein, carbohydrates, B complex of vitamins, vitamin E, iron, trace minerals, and fiber. Cereal grains contain relatively little protein compared to legume seeds, with an average of about 10–12% dry weight. These provide over 200 MT of protein for the nutrition of humans and livestock, which is about three times the amount derived from the more protein-rich (20–40%) legume seeds [3]. Global cereal consumption directly provides about 50% of protein and energy necessary for the human diet, with cereals providing an additional 25% of protein and energy via livestock intermediaries.

In 2004, world cereal production amounted to 1,985 million tones [4]. Major cereal grains produced worldwide include wheat, rice, corn, and barley. Corn, wheat, and rice together account for three-fourths of the world's grain production [5]. Other globally important cereal crops include sorghum, oats, millet, and rye. Asia, America, and Europe produce more than 80% of the world's cereal grains. Wheat, rice, sorghum,

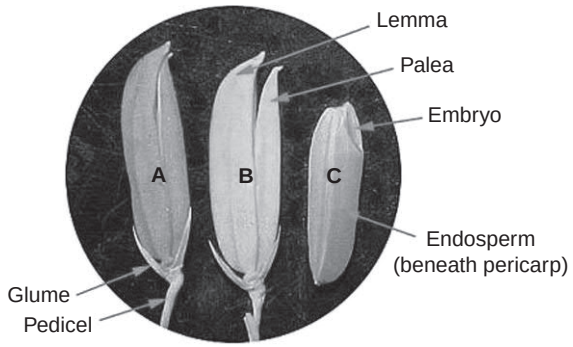

FIGURE 6.1 Rice grain. A: Grain-bearing spikelet showing a pair of slender basal bracts (glumes) and the stalk (pedicel). The inflorescence is composed of numerous spikelets, each bearing a rice grain; B: An empty spikelet with the lemma and palea slightly separated from each other. These two leathery bracts enclosed the grain or caryopsis; C: A grain (caryopsis) removed from spikelet. (From Palomar [66].)

and millet are produced in large quantities in Asia; corn and sorghum are the principal crops in America, whereas barley, oats, and rye are the major crops in the former United Soviet Socialist Republic (USSR) and Europe.

6.1.1.1 Rice (*Oryza sativa* L.)

Rice is a member of the family Poaceae, and it is the major food for about one-third of the earth's population [6, 7]. It has been estimated that 1.7 billion people depend on rice [8]. Cultivated in Asia for thousands of years, rice is also grown in many other parts of the world. Wild rice (*Zizania aquatica*) is native to North America where it was originally harvested from the wild by Native Americans. Another North American wild rice or Indian rice is *Oryzopsis hymenoides*, native to mountains and valleys of Canada and the western United States. Although wild rice is now cultivated, it is expensive and accounts for less than 1% of the American rice market. The rice is first fermented to develop a nutty flavor and to ease hulling.

Rice is harvested with an outer hull or hull intact. This is commonly called rough rice or paddy. The hull, which constitutes about 20% of the weight of rough rice, is made up of the floral envelopes, the *lemma* and *palea*. Brown rice (rice after hulls have been removed) varies from 5 to 8 mm in length. The kernels weigh an average of about 25 mg and are about 2% pericarp, 5% seed coat and aleurone, 2–3% germ, and 89–94% endosperm [9]. The aleurone is the outermost layer of the endosperm. When brown rice is polished to form white rice during milling, the aleurone layer is removed along with the seed coat and pericarp to form the bran. The germ, the pericarp, and the aleurone layer are richer in nutrients as compared to endosperm, and they contain proteins and vitamins. A rice grain is shown in Figure 6.1. Figure 6.2 shows the longitudinal section of a rice grain including the embryo (germ), pericarp (bran), and endosperm.

The embryo or germ is at the upper end. Beneath the brownish outer pericarp and seed coat layers (called the bran) is the endosperm tissue. Most of the vitamin B_1 is found in the germ and bran portions, which are milled off in polished white rice. The detailed structure of rice grain is shown in Figure 6.3, and further details can be found in several publications [5, 8, 10].

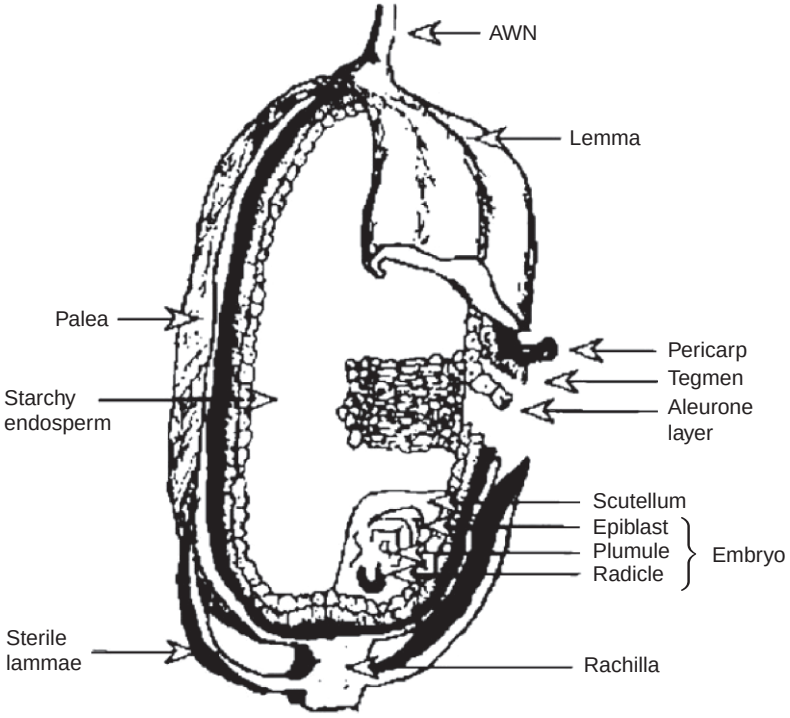

FIGURE 6.2 Longitudinal section of a rice grain. (From Palomar [66].)

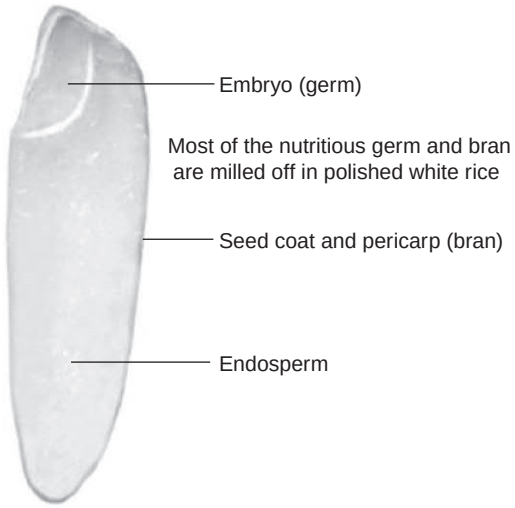

FIGURE 6.3 Structure of a rice grain. (From Haard et al. [2].)

6.1.1.2 Sorghum (*Sorghum bicolor* L. Moench)

Sorghum ranks fourth after rice, corn, and wheat in terms of importance for human nutrition [11]. The plant originates from equatorial Africa and is distributed throughout the tropical, semi-tropical, and arid regions of the world. Today sorghum is an important food crop in Africa [12, 13] and Asia. There are four main types of sorghum-based primarily on how it is used: (i) grain sorghums (including milo), (ii) sweet sorghum or sorgo (used as feed), (iii) Sudan grass (a different but related species), and (iv) broom-corn. The grain is partially covered with glumes, and the most common colors are white, bronze, and brown.

In North America, sorghum is used primarily as livestock feed. Commercial U.S. sorghums are generally 4 mm long, 2 mm wide, and 2.5 mm thick [14]. The kernels are generally spherical, weigh 20–30 mg, and may be white, red, yellow, or brown in color. Hand-dissected kernels are found to be 7.9% pericarp, 9.8% germ, and 82.3% endosperm [15]. The structure of sorghum grain is shown in Figure 6.4, and further details can be found in Hoseney [15]. The outer thick pericarp of a kernel consists of three layers: the epicarp, the mesocarp, and the endocarp. Like corn kernels, sorghum kernels contain both translucent and opaque endosperm.

6.1.1.3 Barley (*Hordeum vulgare* L.)

Barley, like rice and oats, retains its husk (or hull) following harvest. The hull consists of the lemma and palea. Underneath, the 35-mg kernels have the four basic grain components of pericarp, seed coat, germ, and endosperm. The barley kernel is generally spindle-shaped. In commercial varieties grown in the United States, length varies from 7 to 12 mm. A longitudinal section of a barley grain is shown in Figure 6.5.

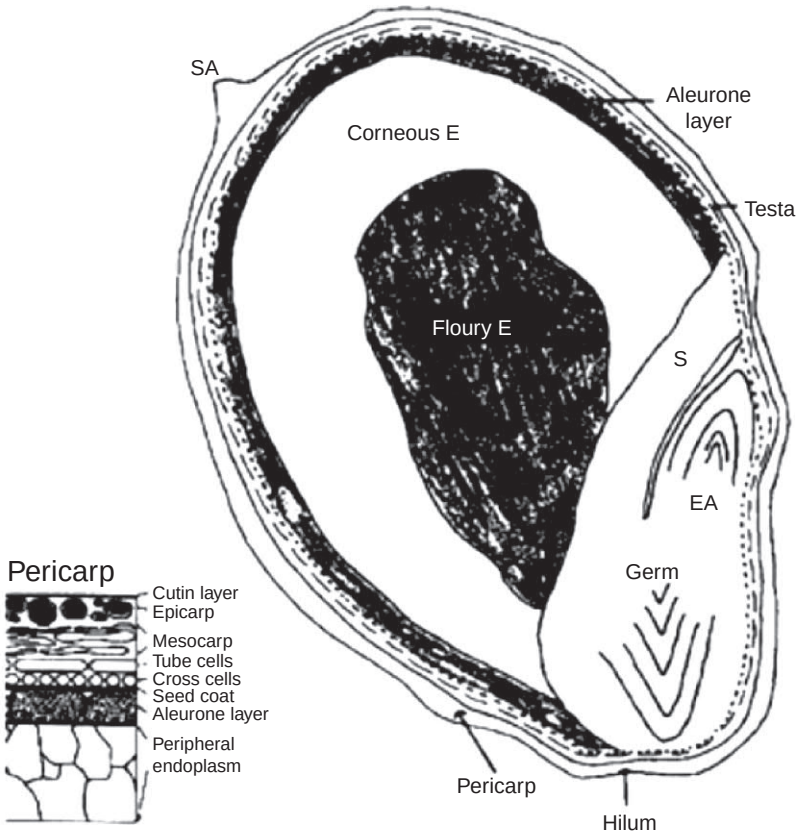

FIGURE 6.4 Diagram of sorghum caryopsis showing the pericarp [cutin, epicarp, mesocarp, tube cells, cross cells, testa, pedicel, and stylar area (SA)], endosperm (E) (aleurone layer, corneous, and floury), and germ [scutellum [4] and embryonic axis (EA) [14].

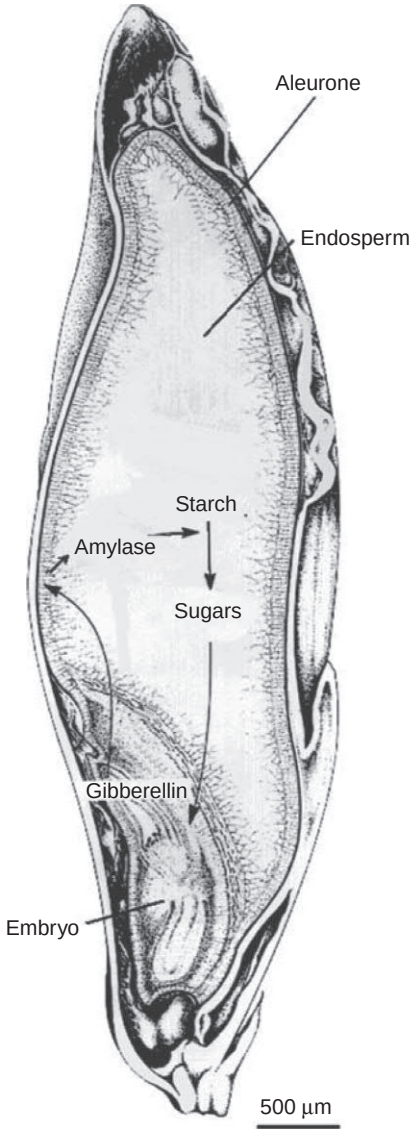

FIGURE 6.5 Longitudinal section of a barley grain. (From Mahapatra and Lan [67].)

The detailed structure of barley grain is shown in Figure 6.6. Further details can be found in MacGregor and Bhatty [16].

6.1.1.4 Wheat (*Triticum aestivum*)

Wheat is native to southwest Asia and the Mediterranean region. Common or bread wheat is widely cultivated in most parts of the world, and is the principal staple food of man. Wheat grains, botanically, are the fruits (caryopsis) of the wheat plant. Mature wheat grain or kernel is roughly ovate- or egg-shaped. The kernels average about 8 mm in length and weigh about 35 mg [15]. The dorsal surface is generally smooth and rounded, but the ventral surface is creased. At the apex, a brush consisting of short hairs is generally present. The color of the kernel varies from dark red through light brown, classed commercially as red wheat, to white; cream or yellow, classed commercially as white, wheat; or amber, in durum wheat.

The wheat kernel structure is shown in Figure 6.7. The kernel consists of three main parts, namely the bran, the endosperm, and the germ or embryo. The outer covering consists of several distinct cell layers in the bran—separated from the flour during most milling processes. It comprises about 12% of the kernel weight. The aleurone, which forms the outer periphery of the endosperm and the innermost layer of the bran, accounts for 3–4% of the weight of the kernel; it is usually removed with the bran during milling. The endosperm consists mainly of starch and makes up about 85 to 86% of the kernel. It is the portion present in white flour. Approximately 2.5–3.5% of a wheat kernel is germ [9], and is separated out in most milling processes. Because it provides nourishment for germination, the germ contains high levels of protein, sugar, and oil. The grain structure of wheat has been documented by Lasztity [17].

6.1.1.5 Corn (*Zea mays* L.)

Corn is the world's most widely grown cereal crop and an essential food source for millions of people in Africa [18], Asia, and Latin America. Corn exists in many varieties and colors, but dent corn is the type used for milling. Corn's flat, broad seeds average around 350 mg, making it the largest of the common cereal seeds. The kernel is made up of three principal parts: hull or bran (pericarp and seed coat), germ, and endosperm. In addition, though, corn kernels frequently have the point of attachment of the cob (tip cap) intact.

Although corn's pericarp and seed coat are collectively called the hull, corn hulls are not the same as the true hulls of rice or barley. Approximately 5–6% of a corn kernel is made up of this outer covering, while 10–14% is germ, with the remainder being endosperm [9]. The outer thin covering is made up of two layers, an outer pericarp and an inner testa or true seed coat. The endosperm consists almost entirely of starch, except in sweet corn. Corn is different from wheat in that both translucent and opaque endosperms are found within a single kernel. Corn kernels or seeds vary in size and shape in different kinds and varieties. The embryo is near one side of the kernel in most kinds rather than in the middle, which contains most of the oil in corn. A longitudinal section of a corn kernel is shown in Figure 6.8, and additional details on the grain structure of corn have been documented by Hoseney [15].

6.1.1.6 Oat

Oats are generally eaten as wholegrain flakes, and the processing contains a heating stage to inactivate enzymes [19]. Most oats are covered in a tough, inedible hull, which must be removed prior to human consumption. The hull contributes to about 30% of the total kernel weight. The oats are called groats after the hulls have been removed [20]. The structure of the oat kernel is shown in Figure 6.9.

Oats, as with barley and rice, retain the hull formed by a floral envelope. Underneath, the oat kernel (called a groat) is similar to wheat or rye. The germ, however, is much larger and narrower than that of wheat, and it extends from 25 to 33% of the length of the groat [9]. The oat groat consists of pericarp, seed coat, hyaline layer, germ, and endosperm. The aleurone makes up the outer layer of the endosperm. Compared with other cereals, oat endosperm contains higher levels of protein and oil. Oat starch is like rice starch in that it exists as

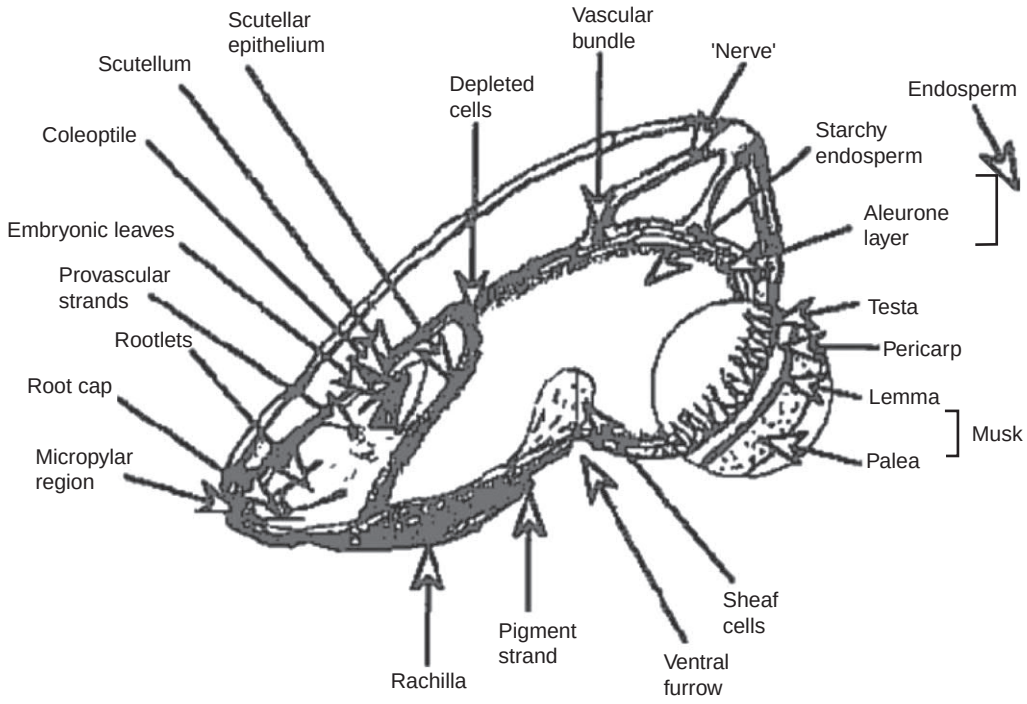

FIGURE 6.6 Structure of barley. (From Haard et al. [2].)

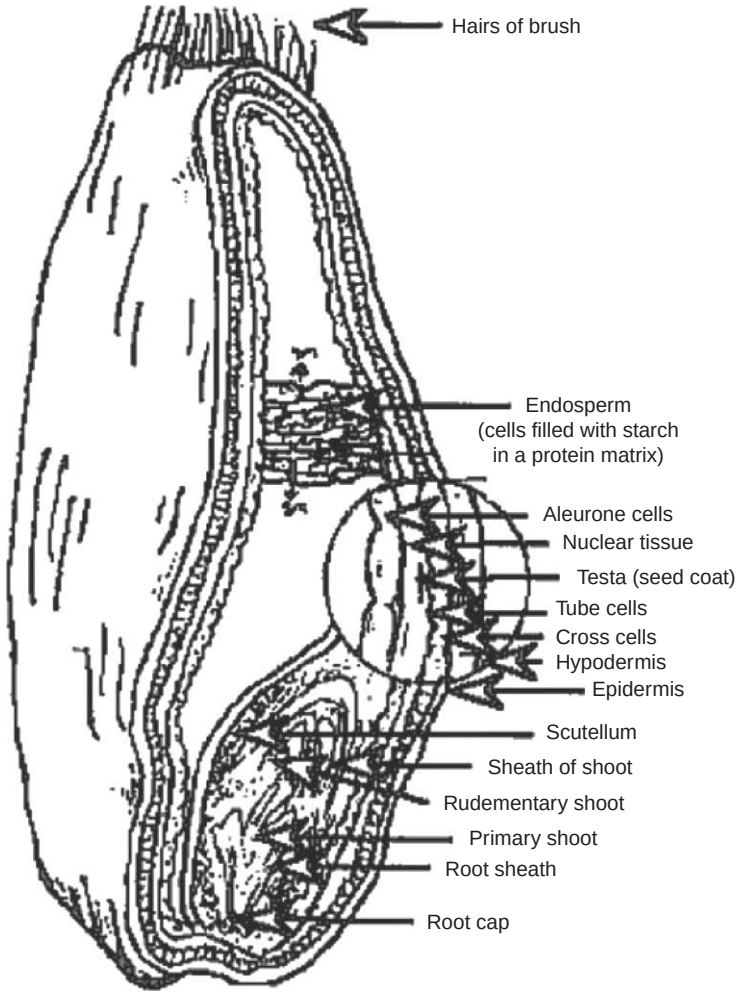

FIGURE 6.7 Structure of wheat kernel. (From Haard et al. [2].)

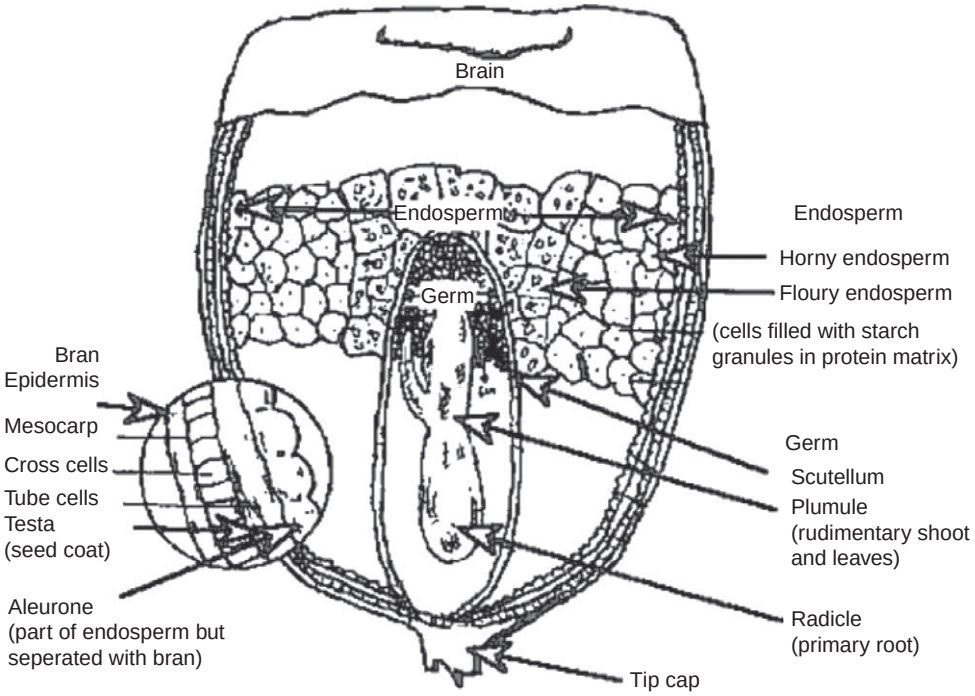

FIGURE 6.8 A longitudinal section of a corn kernel. (From Haard et al. [2].)

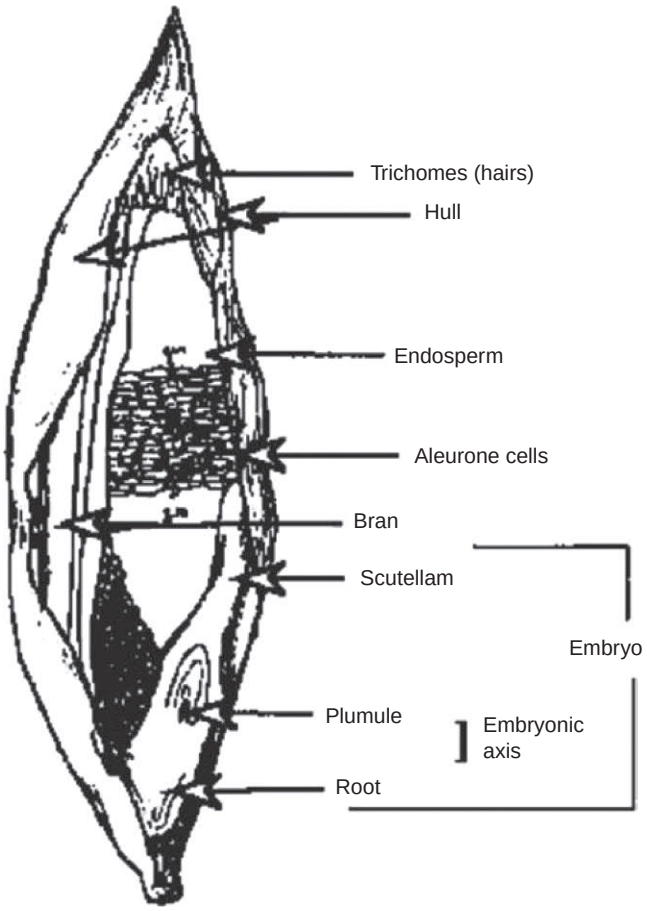

FIGURE 6.9 Structure of an oat kernel. (From Haard et al. [2].)

compound starch granules, which are large granules made up of many smaller individual granules.

6.1.1.7 Rye (*Secale cereale* L.)

Rye is a special European kind of cereal, and more than 90% of the world's production is grown in Europe [21]. Rye kernels are harvested hull-free and have typical grain caryopsis components. The kernels are grayish in color, 6–8 mm in length, and 2–3 mm in width. Like the other cereals, rye consists mainly of pericarp, seed coat, aleurone layer, germ, and endosperm. The endosperm is surrounded by a single layer of aleurone cells. The starch in the endosperm cells has large lenticular and small spherical granules, like wheat and barley. The longitudinal section of a rye grain is shown in Figure 6.10. A microscopic picture of the grain is presented in Figure 6.11.

Before rye grains can be used in food production, the outer part of the grain, the hull, must be removed. After hulling, which generally occurs during threshing, the grains are used whole, cracked, or flaked, or they are ground to make flakes or flour. The starchy endosperm constitutes about 80–85% of the weight of the whole kernel, the germ 2–3%, and the outer layers about 10–15% [22].

6.1.1.8 Pearl Millet (*Pennisetum glaucum*)

Millet and sorghum are often grouped together because their growing conditions, processing, and uses are similar. Pearl millet is one of the two major crops grown in the semi-arid, low-input dryland agriculture regions of Africa and Southeast Asia [23]. Millet was domesticated in Africa, some 3,000 to

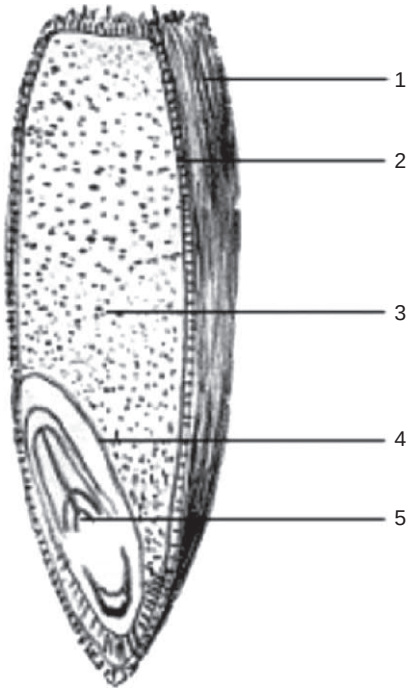

FIGURE 6.10 Longitudinal section through a rye grain [68]. 1: Multilayer husk and seed coat; 2: Aleurone layer; 3: Endosperm; 4: Scutellum; 5: Embryo.

5,000 years ago, and subsequently spread to southern Asia. Pearl millet consists of small (average about 8.9 mg), tear-shaped kernels that are threshed clean of their hulls [15], and, depending on head size, grain number per head ranges from 500 to 3,000 [24]. The caryopsis is very similar to those of other cereal grains. The germ in pearl millet is relatively large (17%) in proportion to the rest of the kernel. Its endosperm has both translucent and opaque endosperm, like those of sorghum and corn. Pearl millet consists of 8.4% pericarp, 75% endosperm, and 6.5% germ [25].

6.1.2 Pulses

Pulses are annual leguminous crops yielding from 1 to 12 grains or seeds of variable size, shape, and color within a pod. Pulses include dry peas, dry beans, vetches, lupins, dry broad beans, lathyrus, lentil, black gram, mung bean, chickpea, pigeon pea, and cowpea, etc. They are used for both food and feed and are important foodstuff in most of the tropical and subtropical countries [26]. The term "pulses" is limited to crops harvested for dry products, excluding, therefore, crops harvested green for forage, used for grazing, or as green manure, and also crops harvested green for food (green beans, green peas, etc.). They also exclude those used mainly for extraction of oil (soybeans and groundnuts), and crops whose

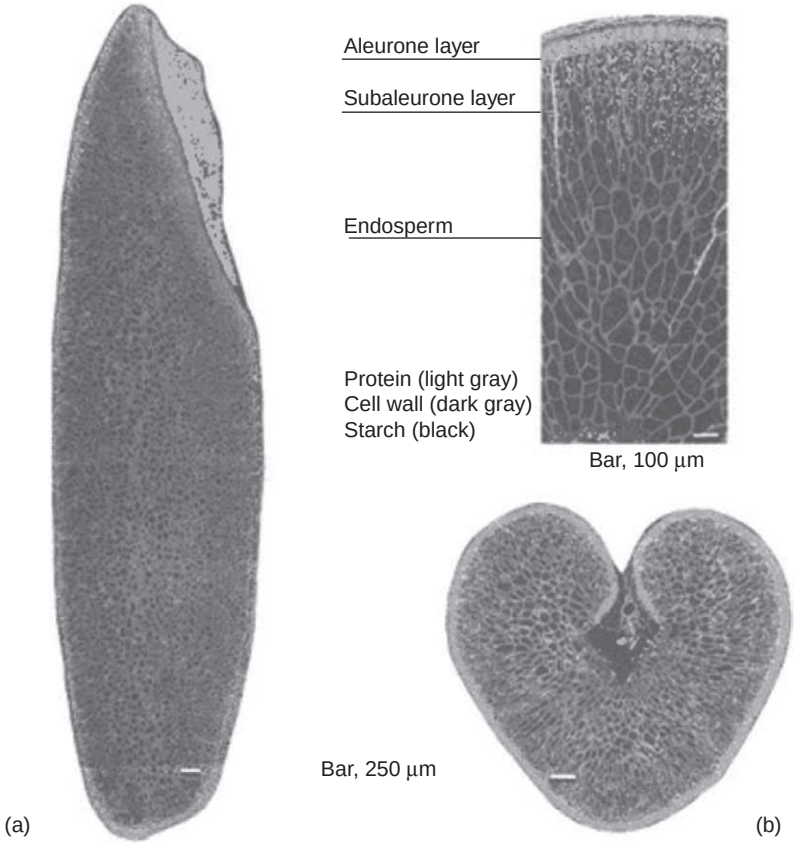

FIGURE 6.11 Microscopic picture of rye grain. (From Nordic Rye Group [22].)

seeds are used exclusively for sowing purposes, such as alfalfa and clover [1].

The structure of food leguminous plants is similar. Mature legume seeds have three major components: the seed coat, the cotyledons, and the embryo axis, which constitute 8, 90, and 2% of the seed, respectively [5]. The structure of a typical legume seed is shown in Figure 6.12. The outer layer of the seed is the testa or seed coat. Usually, legumes have a moderately thick seed coat. In most legumes, the endosperm is short-lived, and, at maturity, it is reduced to a thin layer surrounding the cotyledons or embryo.

6.2 CHEMICAL COMPOSITION

The relative proportions of the main grain components for different grains are presented in Table 6.1. The grain is composed of both organic and inorganic substances, such as carbohydrates, proteins, vitamins, fats, ash, water, mineral salts, and enzymes. Cereal grains are rich in carbohydrates whereas legumes are rich in proteins. The compositions of some of the cereal grains are shown in Table 6.2.

Pulses contain carbohydrates, mainly starches (55–65% of the total weight); proteins, including essential amino acids (18–25%, and much higher than cereals); and fat (1–4%). Pulses are the richest source of vegetarian protein [27–29]. The proportion of seed coat, cotyledon, and embryo in different legumes is presented in Table 6.3. The cotyledons are the major source of nutrients in pulse grains. Legumes contain an appreciable amount of protein and are a good source of minerals. The compositions of some of the Canadian pulses are presented in Table 6.4.

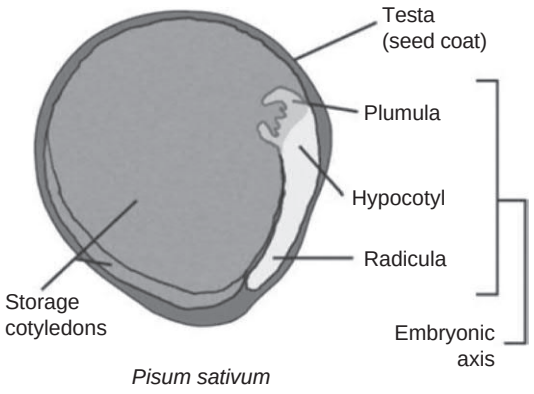

FIGURE 6.12 Drawing of a mature pea (*Pisum sativum*) seed, a typical non-endospermic seed with storage cotyledons and the testa. (From Salunkhe et al. [69].)

TABLE 6.1
Grain Size and Proportions of the Principal Parts of Mature Kernels

Cereal	Germ,%	Pericarp,%	Aleurone,%	Endosperm,%
Barley	3.4	18.3	—	79
Corn	2.7	7.9	6.7–7.0	81–84
Oat	3.7	28.4–41.4	—	—
Pearl Millet	17.4	7.5	—	75
Rice	3.5	1.5	4–6	89–94
Rye	3.5	12.0	—	85
Sorghum	7.8–12.1	7.3–7.9	—	80–85
Wheat (bread)	2.7	7.9	6.7–7.0	81–84
Wheat (durum)	1.6	12.0	—	86

Source: Hoseney and Faubion [70].

TABLE 6.2
Proximate Composition of Cereal Grains, % Dry Weight

Cereals	Crude Protein	Crude Fat	Ash	Crude Fiber	Carbohydrate
Barley	11.0	3.4	1.9	3.7	55.8
Corn	9.8	4.9	1.4	2.0	63.6
Oats	9.3	5.9	2.3	2.3	62.9
Pearl millet	11.5	4.7	1.5	1.5	63.4
Rice, brown	7.3	2.2	1.4	0.8	64.3
Rye	8.7	1.5	1.8	2.2	71.8
Sorghum	8.3	3.9	2.6	4.1	62.9
Wheat	10.6	1.9	1.4	1.0	69.7

Source: Alais and Linden [71].

TABLE 6.3
Proportion of Seed Coat, Cotyledons, and Embryo for Some Selected Legumes

Legume	Seed Coat, %	Cotyledon, %	Embryo, %
Cowpea	10.64	87.23	2.13
French bean	8.64	90.37	0.99
Lentil	8.05	89.97	1.98
Mung bean	12.09	85.61	2.30
Peas	10.00	89.28	1.72
Pigeon pea	15.50	83.00	1.50

Source: Leubner [72].

TABLE 6.4
Composition of Some of the Canadian Pulses

Pulses	Protein	Fat	Ash	Fiber	Starch
Bean	25.1	1.5	4.3	15.3	38.0
Chickpea (desi)	23.0	5.4	3.2	25.9	36.4
Chickpea (kabuli)	24.4	5.9	3.2	8.7	41.1
Field pea	23.7	1.3	2.8	16.6	45.5
Lentil	26.3	1.1	2.8	13.6	45.0

Source: Wang and Daun [73].

6.3 GRAIN-GRADING SYSTEMS

Grain-grading systems used around the world are similar and depend mainly on visual inspection and comparison of samples [30]. In Canada, grain is graded on five factors, namely test weight, varietal purity, soundness, vitreousness, and maximum limit of foreign material (not including dockage). Of these, the latter four factors are determined visually by trained personnel, and thus can be influenced by experience and human fatigue [31]. In Australia, grain is routinely segregated based on moisture and protein contents determined by near-infrared spectroscopy [32]. Despite training, the grading decisions are inherently subjective and are influenced by the individual experience of a grain inspector [30].

The grading factors in European Union countries include moisture, broken kernels, grain besatz (shrunken kernels, other grains, insect-damaged kernels), sprouted kernels, black besatz (wheat seed, ergot, unsound grain, chaff, impurities), and hectoliter weight. In Russia, color, odor, taste, moisture, foreign kernels, and damaged kernels are listed as grading factors [21].

6.3.1 Recent Progress in Grain-Grading Technology

Research has been carried out to replace the tiresome job of visually inspecting grain samples by a machine vision system (MVS). Such an MVS should be capable of identifying and grading the grain on the basis of size and shape [33], color [34, 35], and texture [36]. Color and size are important grading factors for certain commodities such as peas and chickpeas.

Several systems have been reported in the literature for inspecting grains using machine vision [37, 38]. For classification purposes, shape is the best feature, followed by color and length of the kernels [31]. Visual appeal directly influences consumer acceptance and hence value of the grains. The main visual factor is the color of grain, which is directly related to its market value [39]. Some investigations have been carried out using color features [40] for classifications of different cereal grains and their varieties and for correlating vitreosity and grain hardness. Liu and Paulsen [41] measured the whiteness of corn quantitatively by computer vision. This is a useful application as the prices of corn depend on color [42].

In recent years, optical, mechanical, and electrical techniques have been applied to rapid grain grading and classification [43]. Delwiche et al. [44] using near-infrared spectroscopy with an artificial neural network identified hard red winter and hard red spring wheat classes with accuracies of 95 to 98%. Steenhoek et al. [45] developed a computer vision system to evaluate blue-eye mold and germ damage in corn grading. Sapirstein et al. [46] employed image processing to identify grains such as wheat, oats, barley, and rye. Gunasekaran et al. [47] detected stress cracks and other damage in corn kernels and soybeans from their images. Shatadal et al. [48] used parameters such as area, length, width, and compactness of grain binary images to recognize wheat, oats, barley, and rye. Zayas et al. [49] determined wheat variety by using the texture characteristics of wheat images. The Canadian Grain Commission (CGC) has developed a grading instrument for assessing the color and size distribution of lentils. Modules for grading other pulse grains such as peas, chickpeas, and beans are under development [50].

Stress fissures detection is one of the most important tasks in rice grain quality inspection. A machine vision system has been used successfully to reveal fissure lines in rice [51]. Peck damage is another quality factor affecting the grading and marketing of rice. The rice stink-bug, *Oebalus pugnax*, is a key pest that causes peck damage. An objective method has been developed to classify pecky rice kernels [52].

6.3.2 Grading Systems

Grading systems are used in the marketing of grain. The grade standing systems are not universal and vary significantly between countries. The U.S. grading system for yellow corn is presented in Table 6.5. The grading systems for wheat in Germany and Italy are presented in Tables 6.6 and 6.7, respectively.

TABLE 6.5
U.S. Grade Requirements for Yellow Corn

U.S. Grade	Minimum Test Weight/Bu, lb	Heat Damaged Kernels, Maximum Limit, %	Total Damaged Kernels, Maximum Limit, %	Broken Corn and Foreign Material
No. 1	56.0	0.1	3.0	2.0
No. 2	54.0	0.2	5.0	3.0
No. 3	52.0	0.5	7.0	4.0
No. 4	49.0	1.0	10.0	5.0
No. 5	46.0	3.0	15.0	7.0
U.S. sample grades*	n/a	n/a	n/a	n/a

Source: Bakker-Arkema [74].

* U.S. sample grade is corn that does not meet the requirements for U.S. Grade Nos. 1, 2, 3, 4, or 5; contains eight or more stores that have an aggregate weight in excess of 0.20% of its sample weight, or two or more pieces of glass; has a musty, sour, or commercially objectionable foreign order; or is heating otherwise of distinctly low quality.

TABLE 6.6
Wheat Classification System in Germany

	Quality Class			
Parameter	**Elite E**	**High Quality A**	**Normal B**	**Soft K**
Protein (%) min.	13.8	13.2	12.8	12.4
Sedimentation (ml) min.	47	33	26	19
Flour yield (%) min.	76	74	74	76
Water absorption (%) min.	56.9	55.9	53.7	52.6
Falling number (sec) min.	285	255	255	235
Loaf volume (ml/100g) min.	710	650	590	560

Source: Lasztity and Salgo [21].

TABLE 6.7
Italian System of Classification of Common Wheat

	Alvo-Graph		Protein N × 5.7	Farinograph Stability	Falling Number
Class	**W**	**P/L**	**(%)**	**(MIN)**	**(SEC)**
Improver	300	1	14.5	15	250
High quality	220	0.6	13.5	10	220
Normal bread wheat	160	0.6		5	220
For confectionary products	115	0.5			240

Source: Lasztity and Salgo [21].

6.3.3 Computer Vision Technology

A fast and objective grain-grading and classification system would reduce the inaccuracy caused by inspector subjectivity. Grain quality inspection using computer vision is a nondestructive method. However, the application of computer vision technique for the objective classification of cereal grains and varieties is still at its infant stage. Further development in both software and hardware is necessary for this technology to become more widely used.

6.4 HARVESTING AND THRESHING

Harvesting of cereal grains and pulses refers to the activities performed to obtain the kernels of the plant for grain, or the entire plant for forage and/or silage uses. These activities are accomplished by machines that cut, thresh, screen, clean, bind, pick, and shell the crops in the field. Harvesting also includes loading harvested crops into trucks and transporting crops in the grain field [53].

Cereal kernels are cut as close as possible to the inflorescence. This portion is threshed, screened, and cleaned to separate the kernels. The grain is stored in the harvesting machine while the remainder of the plant is discharged back onto the field. Combines perform all the above activities in one operation. Large amounts of abrasion and breakage may occur as a result of threshing, augering, and impaction when harvested by combines, followed by transfer from combines to grain trucks with subsequent transfer to farm storage [54]. Binder machines only cut the grain plants and tie them into bundles, or leave them in a row in the field, called a windrow. The bundles are allowed to dry for threshing later by a combine with a pickup attachment.

Corn is harvested by mechanical pickers, picker/shellers, and combines with corn head attachments. These machines cut and husk the ears from the standing stalk. The sheller unit also removes the kernels from the ear. After husking, a binder is sometimes used to bundle entire plants into piles (called shocks) to dry [53]. For forage and or/silage, binders, crushers, field choppers, mowers, windrowers, and similar cutting machines are used to harvest grasses, stalks, and cereal grains. These machines cut the plants as close to the ground as possible and leave them in a windrow. The plants are later picked up and tied by a baler. Harvested crops are loaded onto trucks in the field. Grain kernels are loaded through a spout from the combine, and forage and silage bales are manually or mechanically placed in trucks. The harvested crop is then transported from the field to a storage facility [53].

6.4.1 Combine Harvester

Grain harvesting and handling procedures have undergone dramatic changes, particularly with the development of the combine harvester. The combine harvester has become the standard for harvesting grain in the industrialized countries [55] and can perform labor-intensive operations such as harvesting and threshing simultaneously at the field level. Combines are able to fill their grain tanks within 12 minutes and can off-load to a grain cart in as little as 2.5 minutes [56].

6.4.1.1 Function of Combine Harvester

The main process functions of a combine harvester (Figure 6.13) consist of reaping, threshing, separating, and cleaning (Figure 6.14). The threshing function especially is very important because the quality of harvested grain into the tank of the combine harvester depends mainly on the ability of this function [57].

6.4.1.2 Performance of a Combine Harvester

The performance depends on its design, the settings of the individual working elements, the experience of the operator, the harvest conditions, and very strongly on the properties of the harvested materials (Figure 6.15). The technical equipment includes the design of the combine, the threshing and separating system, the dimensions of the individual elements, and engine power. The settings of elements may consist of the speed of the working elements and the concave clearance. Harvest conditions are influenced mostly by the weather, topography, soil conditions, and field size. Though operators are supported by various electronic systems, operators' experience helps in reaching a high field performance. Grain

FIGURE 6.13 Combine harvester. (From Miyamoto and Murase [57].)

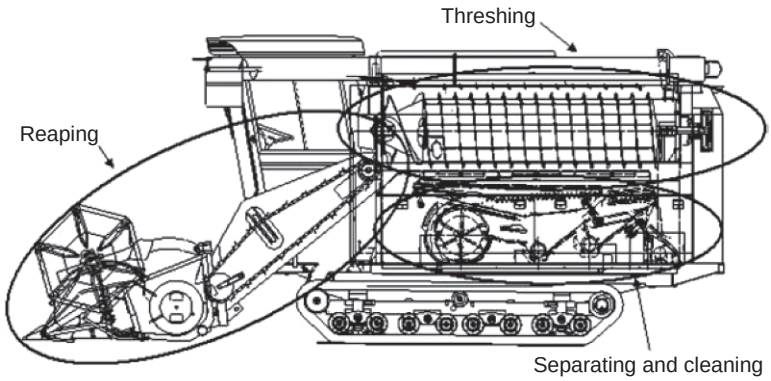

FIGURE 6.14 Main process functions of combine harvester. (From Miyamoto and Murase [57].)

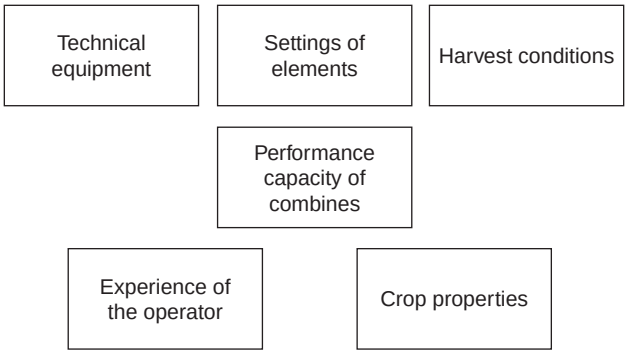

FIGURE 6.15 Influences on the performance capacity of combines. (From Wacker [55].)

properties strongly influence performance capacity and so the field performance [55].

6.4.2 Whole-Crop Harvesting System

Conventional combine harvesting systems harvest only grains and leave the straw and chaffs on the field. If the crop residues are utilized for bioenergy production they have to be harvested with extra cost. Recently, the utilization of crop residues for bioenergy production increased the monetary value of crop residues [58]. Hence, there is a need to harvest both cereal grains as well as crop residues for maximizing profit.

In the case of a whole-crop harvesting system, the whole crop is harvested and transported to a centrally located processing unit where the crops are separated into grains, straws/stover, and chaffs. The cereal grains can be supplied to processing and storage facilities, and straws with high moisture content can be processed into different raw materials based on the requirement of biorefineries.

In the McLeod harvest system, developed by Bob McLeod in western Canada, the harvesting unit cuts the whole crop and threshes to separate the graffs (grains, chaffs, and weed seeds) from the straw [58]. The straws are left on the field for further drying and baling operation. The graff is transported to a central location, where it is further processed to separate grains from chaffs and weed seeds by a milling unit. Figure 6.16 shows the sequential operation of the McLeod harvesting system. It has been reported that the McLeod system is more profitable than the conventional harvesting system.

As new markets begin to emerge for straw and chaff, such as ethanol production and strawboard manufacturing, innovative methods of whole-crop harvesting are being proposed. Five methods of harvesting such as windrow/combine,

straight-cut, stripper header, the McLeod system, and whole-crop baling have been modeled and compared by Ragan [59].

6.4.3 Windrow/Combine

The crop is windrowed (swathed), then harvested with a standard combine with a pick-up header. Straw and chaff are spread behind the combine or dropped in a windrow for baling later. As the system involves separate cutting and threshing operations, additional power is required to pass the entire crop through the combine. Hence, energy consumption is substantially higher than for other systems, and the combine work rate is slow because it is processing nearly all of the available plant material [59]. The conventional swath and combine harvesting method is shown in Figure 6.17.

6.4.4 Straight Cut

The straight-cut system requires one less field operation than the windrow/combine system resulting in less time in the field and lower energy consumption. However, the straight-cut system cannot be used with crops such as pulses and oilseeds. The longer stubble resulting from the higher cut may reduce efficiency in chaff and straw recovery [59]. Figure 6.18 shows the conventional straight-cut harvesting method.

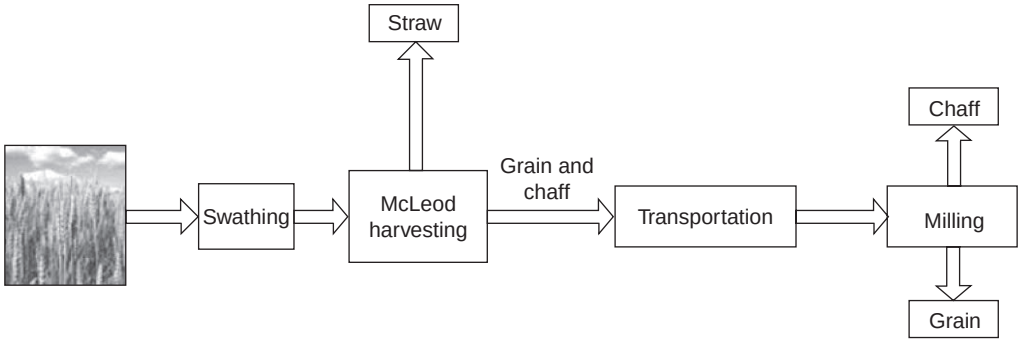

FIGURE 6.16 Sequential operation of McLeod harvesting system. (From Sokhansanj et al. [58].)

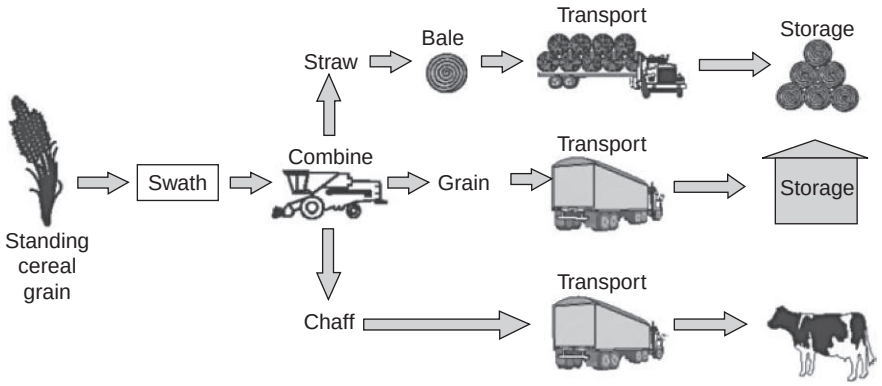

FIGURE 6.17 Conventional swath and combine harvesting method. (From Ragan [59].)

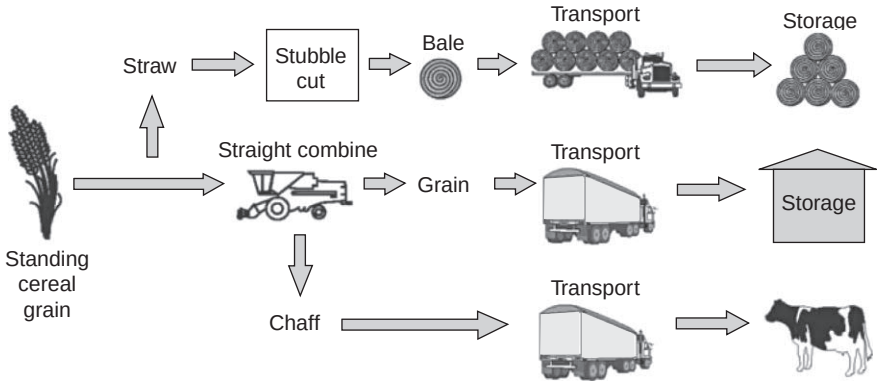

FIGURE 6.18 Conventional straight-cut harvesting method. (From Ragan [59].)

6.4.5 Stripper Header

Currently, stripper headers are being used on conventional combines in place of the straight-cut or pick-up header. Most of the threshing is done by the header, and as a result, much of the capacity of the combine itself is not used. A specifically designed harvesting machine would need to be developed to operate with the stripper header in order to fully capture its inherent advantages.

The stripper header is a very efficient method of collecting grain because of its low energy needs and the short time required for harvesting. In effect, the stripper header removes the heads from the crop and leaves the straw standing. A separate operation is then required to cut the straw so other field operations such as seeding can be conducted with a minimum of problems. Also, some of the straw is flattened in the harvesting operation, making it more difficult to recover later. A limitation is that the stripper header may only be used efficiently with cereal grains [59]. The stripper header harvesting method is shown in Figure 6.19.

6.4.6 The McLeod System

This system is being developed by Bob McLeod of Winnipeg. It consists of a standard combine straight-cut header with a feeder housing and threshing cylinder. The grain and chaff are collected together in a large hopper, and the straw passes over a set of straw walkers to collect any free grain. The unit would be pulled by a large two-wheel drive tractor. The grain/chaff mixture would be transported to the farmyard where it would be processed further by an electric motor driven unit already developed by McLeod [59]. Figure 6.20 shows the McLeod harvesting method, using a straight-cut header.

6.4.7 Whole-Crop Baling

This model does not yet exist as an entire system. It has been based partly on existing machinery—swather and baler—and on assumptions based on previous research done to determine if crops could be baled and then threshed without loss of grain quality or quantity. First, the crop would be swathed to a stubble height of eight inches. Then the entire unthreshed crop would be baled with a medium-sized round baler and transported to the farmyard. In the yard, the bales would be unwrapped and fed through a stationary processor that would perform all the functions of a normal combine [59]. The whole-crop baling method is shown in Figure 6.21.

6.4.8 Stripper Harvester

Stripping involves combing the grain from the panicles without cutting the straw. The simplicity of operation and reduced fuel bills make stripper harvesting an attractive alternative method to conventional harvesting methods [60]. The working part of the header is a rotor of flexible teeth with keyhole access between each pair of teeth. The rotor rotates in the direction opposite to that of a standard reel, and as the crop heads pass through the recesses, the seeds and chaff are stripped from the plant. A conveyor and auger convey the grain and chaff into the combine. It is suitable only for use with grains such as wheat, barley, and oats, and similar types of plants [59].

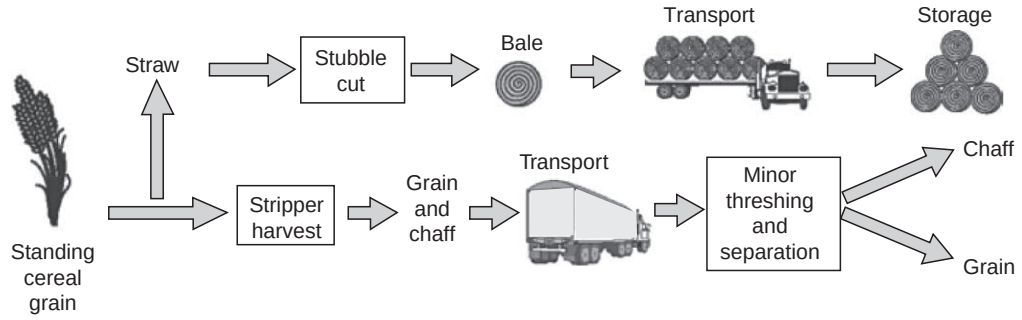

FIGURE 6.19 Stripper header harvesting method. (From Ragan [59].)

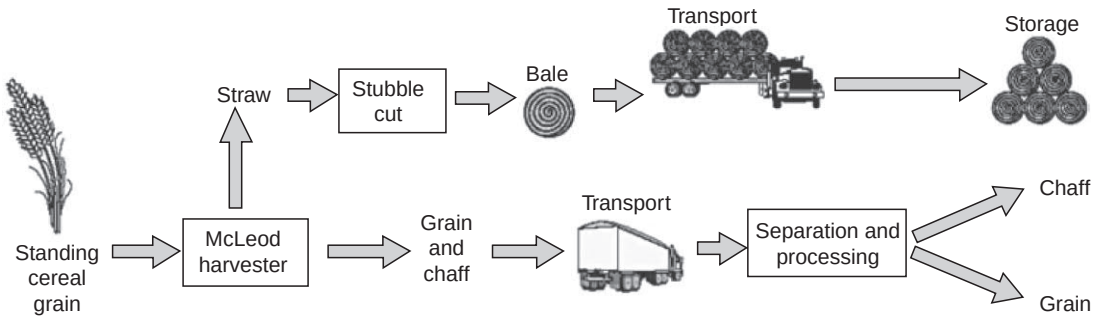

FIGURE 6.20 McLeod harvesting method, using straight-cut header. (From Ragan [59].)

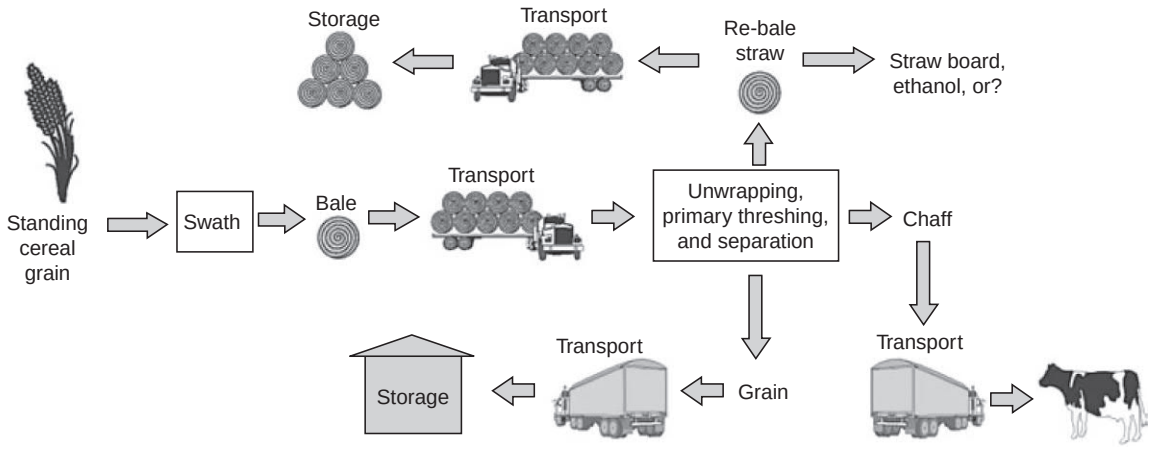

FIGURE 6.21 Whole-crop baling method. (From Ragan [59].)

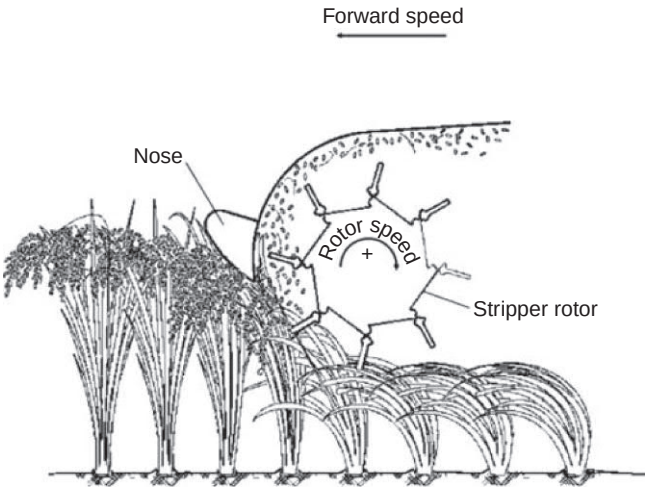

FIGURE 6.22 Principle of operation of the Silsoe stripper rotor. (From Tado and Quick [60].)

With the stripper header in operation, the grain and some leaves are the primary parts of the plant that pass through the thresher. The entire stem of the plant is left standing in the field. The amount of biomass left standing by these headers is greater than that of conventional combine headers. Harvesting speeds could be as high as 2.3 m s^{-1} (5 miles hr^{-1}) with this type of header, but they do not work efficiently in lodged rice [61].

The most promising stripper system header was developed by Silsoe Research Institute, UK. The Silsoe stripper rotor (Figure 6.22) combs up through the crop as the machine moves forward removing the grain from the plant in situ. Flexible V-shaped teeth guide the plant into a keyhole-shaped slot at the base of the teeth where stripping occurs. The upward rotation of the stripper teeth with respect to the crop allows the rotor to pick up fallen plants or lodged crops. The rotor is enclosed on the top by a hood that guides the stripped material back for collection or further processing. A nose protrudes

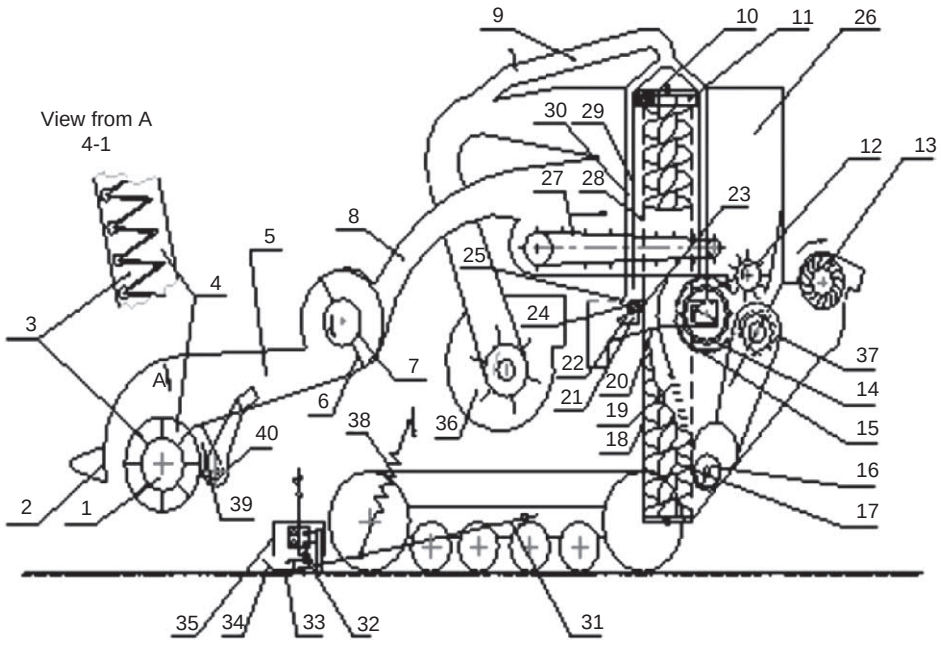

FIGURE 6.23 Schematic arrangement of combine stripper harvester for rice and wheat. (From Jiang et al. [64].)

forward from the inlet edge of the hood to deflect gently the crop prior to stripping. The stripper rotor simultaneously carries out four functions: crop lifting, harvesting, partial threshing, and crop transport that corresponds to the function of the crop reel, cutter bar, threshing drum, and crop conveyor on a conventional combine harvester [62]. Combines fitted with stripper headers are used in the rice fields of Australia and the United States [63]. Specifically designed equipment to complement the stripper header has not yet been developed [59].

A new combine harvester that is capable of cutting and windrowing straw immediately after stripping has been developed for rice and wheat harvesting [64]. Stripper header, grain collection and cleaning mechanism, and straw cutting and windrowing are key components of the combine harvester. The field-testing results show that the free grain loss is low owing to the use of a pneumatic conveying system, and high grain cleaning capacity is achieved by using a vertically cylindrical cleaning system. The schematic diagram of the combine stripper harvester is shown in Figure 6.23. Stripper headers have become more popular for harvesting rice. The shortcomings of existing strippers include high shatter losses caused by crop disturbance, incomplete grain detachment, failure to collect detached grains, blockages of essential crop aligning or stripping components, and inability to harvest tangled and lodged crops satisfactorily [65].

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7 Postharvest Handling of Grains and Pulses

Ajit K. Mahapatra and Yubin Lan

CONTENTS

7.1 STORAGE OF GRAINS AND PULSES

7.1.1 Grain Storage: Perspectives and Problems

The primary aim of storage is to prevent deterioration of the quality of the grain. This is achieved through control of moisture and air movement, and by preventing infestation of microorganisms, and attacks of insects and rodents. Foodgrains can be stored for relatively long periods of time under proper storage conditions (low temperature, inert atmosphere, etc.), with little or no detectable loss of quality. The length of time grain can be held in storage depends on the moisture content of the grain, the temperature of the grain, and whether the grain can be kept from heating by means of aeration [1]. Grain spoilage is the result of microorganisms (bacteria, yeast, and fungi) using grain nutrients for growth and reproductive processes. Microorganisms also produce heat during growth that can increase the

temperature of stored grain. Under proper environmental conditions, certain microorganisms can produce toxins or other products that can cause serious illness and even death when consumed by livestock or humans [2].

Safe storage must maintain grain quality and quantity. This means protecting it from weather, molds and other microorganisms, the addition of moisture, destructively high temperatures, insects, rodents, birds, objectionable odors, and contamination. High temperature and high moisture are the most significant factors affecting grain quality in storage. Each can cause rapid decline in germination, malting quality, baking quality, color, and oil composition. Insects and molds impair the quality of grain directly by their feeding and development, and indirectly through the generation of heat and moisture. High temperatures and moistures favor the development of insects and molds. The development of insects is limited by temperatures below 15°C and by moistures below 9% in cereal grains, whereas development of molds is limited by temperatures below 10°C and by moistures below 13% in cereal grains. Spraying with insecticides or fumigating minimizes insect problems but leaves chemical residues in grain [3].

7.1.1.1 Environmental Factors Influencing Grain Quality

To store grain successfully, grain and the atmosphere in which it is stored must be maintained under conditions that discourage or prevent the growth of microorganisms that cause spoilage. Stored-grain ecosystems are complex due to a large number of abiotic and biotic factors and their interrelationships [4]. Computer models have been used to simulate abiotic factors, such as temperatures, moisture contents, and gas concentrations, and biotic factors, such as population dynamics of insects and mites, and fungal growth [5]. Figure 7.1 shows the essential elements of the storage ecosystem, the boundaries of which are the storage container (bag, drum, silo, warehouse, or whatever).

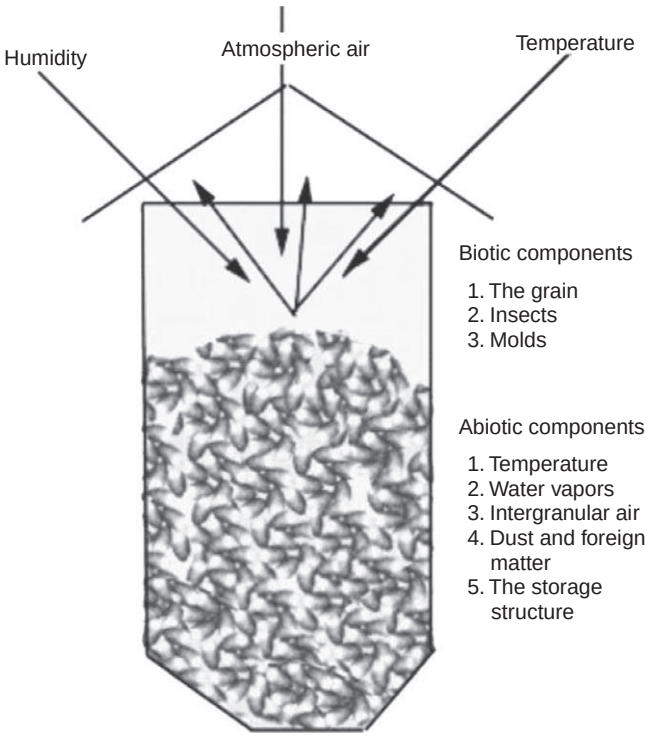

FIGURE 7.1 Essential elements of storage ecosystem. (From Agricultural Research Station of Israel [75].)

7.1.1.2 Types of Storage Facilities

Storage facilities take many forms, ranging from piles of unprotected grain on the ground, underground pits or containers, and piles of bagged grain, to storage in bins of many sizes, shapes, and types of construction. Some of the storage facilities used in the rural areas of Bangladesh are shown in Figure 7.2.

FIGURE 7.2 Rural storage structures in Bangladesh. (From ASB [76].)

7.1.1.2.1 On the Ground

Grain is normally piled on the ground unprotected only between harvest and the availability of transport equipment. Losses are smaller if stored for short periods of time, but the grain is exposed to rodents, birds, insects, and wind so that losses become severe within a week. The angle of repose, pile radius, and bushels for corn, wheat, and sorghum piled from 50 and 60 feet heights for cone-shaped outdoor grain piles are shown in Table 7.1. The amount of ground surface area required for piles that are 15, 20, and 25 feet high for wheat, corn, and grain sorghum are presented in Table 7.2.

If grain must be piled outside on the ground, drainage is a crucial factor. The pile should be on high ground and the earth crowned under the pile. Placing plastic on the ground absolutely is essential to keep soil moisture from migrating into the grain. Piles can be covered with plastic or a tarp to reduce wetting by rain and snow and to minimize damage by wind and birds. Air must flow near the plastic cover to reduce condensation and carry the moisture away. If a rectangular pile is made to store grain, orient the pile north and south to allow the sun to dry condensation off the sloping sides of the cover [1]. The average filling angles of some grains are given in Table 7.3. The quantity of grain in piles can be estimated using Table 7.4. Grain piled outdoors is shown in Figure 7.3.

7.1.1.2.2 Underground

Underground pits (Figure 7.4) are an effective, low-cost method of long-term grain storage, and are most commonly used for storing drought feed reserves on farms. Feed grain

TABLE 7.1
Angle of Repose, Pile Radius, and Bushels for Some Selected Grains Piled from 50 and 60 Foot Heights

Height (ft)	Grain	Angle of Repose (°)	Pile Radius (ft)	Bushels
50	Corn	22	124	644,004
	Sorghum	27	98	402,251
	Wheat	25	107	479,526
60	Corn	22	148	1,100,000
	Sorghum	27	118	696,272
	Wheat	25	129	832,581

Source: Herrman et al. [86].

TABLE 7.2
Pile Width (ft) and Bushels for One-Foot Length of Elongated Triangular-Shaped Outdoor Grain Piles

Pile Height (ft)	15		20		25	
Grain	Width	Bu/ft	Width	Bu/ft	Width	Bu/ft
Corn	74	445	99	792	124	1237
Sorghum	58.9	353	78.5	628	98	981
Wheat	64	386	85.8	686	107	1072

Source: Herrman et al. [86].

TABLE 7.3
Average Filling Angle of Some Selected Grains

Grain	Average Filling Angle (°)
Barley	28
Corn (shelled)	23
Oats	28
Sorghum	29
Soybeans	25
Sunflower (nonoil)	28
Sunflower (oil)	27
Wheat (durum)	23
Wheat (hard red spring [HRS])	25

Source: NDSU [87].

TABLE 7.4
Approximate Capacities of Unconstrained Grain Piles

Pile Height (ft)	Pile Diameter	Total Bushels	Bushels (Additional 1 ft of Pile Length)
3	12.9	105	15
4	17.2	250	28
5	21.5	480	43
6	25.7	840	62
7	30.0	1330	85
8	34.3	1980	110
9	38.6	2820	140
10	42.9	3870	170
11	47.2	5150	210
12	51.5	6700	250
13	55.8	8500	290
14	60.0	10,600	340
15	64.3	13,000	390
16	68.6	15,900	440
17	72.9	19,000	500
18	77.2	22,500	560
19	81.5	26,500	620
20	85.8	31,000	690

Source: NDSU [87].

has been recovered in good condition after more than 10 years. The main drawback of underground storage is the difficulty of removing grain. Grain moisture content must be less than 12% to keep the risk of spoilage low. The pit should be located on a well-drained site above the water table, with the immediate surrounds graded to prevent rainfall runoff collecting in the pit area. Water seepage through the sidewalls of the pit is a major concern. Pits should be kept at least 10 m apart to prevent seepage from an empty pit to a full one, and the pit should not be more than 3 m wide. Well-Constructed pit storage is air-tight and oxygen levels gradually reduce over time. The low oxygen levels prevent development of damaging numbers of grain insects. Grain protectants can be applied to the grain when it is placed in storage [6]. Underground storage protects the grain from variations in temperature; the construction is relatively simple; and it protects grain from insects and molds because of the low oxygen and high CO_2 content of the interseed air [7].

7.1.1.2.3 Bagged Storage

Bagged grain can be stored in almost any shelter that protects the bags from weather and predators, and bags can be handled without any equipment. The advantages of a bagged storage system are the lower capital costs without any need for sophisticated aeration and fumigant circulation facilities [8]. However, both bags and bag storage space become expensive, particularly where manpower costs are high. Dunnage should be used to keep grain bags at least 15 cm off the floor. Stack size should not exceed 6 × 9 m, and a stack should be divided into six blocks containing 256 bags, with a row of 6 bags lengthwise adjacent to a row of 10 bags widthwise [9]. Another temporary storage option that holds some promise is the use of large plastic silage bags. Grain going into these bags should be dry and cool (under 15% and 60°F). A system specifically designed for handling dry grain is available from the manufacturer that reportedly greatly reduces grain damage [1]. A bagged storage facility is shown in Figure 7.5.

7.1.1.2.4 Bulk Storage

Bulk storage in beans is the most widely used type of storage for cereal grains. Bins are constructed of steel, aluminum, concrete, and even wood or plywood. Round bins are most common, but large, flat storage buildings are used as well. Figures 7.6 to 7.12 show large-scale grain storage facilities. The capacity of a grain bin per unit depth is presented in Table 7.5.

FIGURE 7.3 Grain piled outdoors. (From Agriculture and Agri-Food Canada [77].)

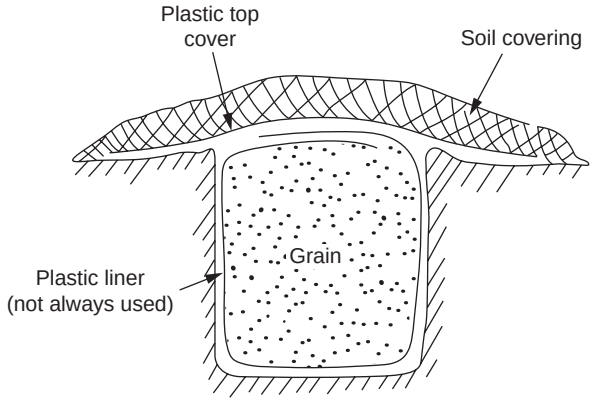

FIGURE 7.4 Underground storage pit. (From DPIF [6].)

FIGURE 7.5 A bagged storage facility. (From Agriculture and Agri-Food Canada [77].)

7.1.1.3 Insects

Insects are a major problem for the storage of grains and seeds. Not only do insects consume some of the grain, but they also contaminate the grain. The U.S. Department of Agriculture (USDA) has estimated that storage losses due to insects exceed $470 million per year [7]. Insects that can live on grain can be divided into those that develop within grain kernels (granary weevils, rice weevils, corn weevils, lesser and larger grain borers, and Angoumois grain moths) and those that develop outside the kernels (red flour and rusty grain beetles, sawtoothed grain beetles, cadelles, khapra beetles, and Indian-meal moths). Some of the insects are shown in Figure 7.13. Grain-damaging insects multiply slowly or not at all below 16°C (60°F), and they cannot survive in temperatures of 42°C (–107°F) or above [10]. Navarro, Noyes, Armitage, and Maier [11] stated that stored-grain insects develop well at 27–34°C (81–93°F) and thrive best at about 29.5°C (85°F).

Moisture is another important factor in controlling grain infestation. Generally, moisture contents of 9% or lower restrict infestation. Even though the grain is stored at a relatively safe storage moisture of 11–14%, in the presence of insects the grain often "heats." The heat is caused by the metabolic heat of the insects. Because of the increased temperature, moisture migration occurs and results in increased moisture in pockets of grain. This leads to microorganisms growing. Air movements in grain and development of hotspots due to insect infestation during storage are shown in Figures 7.14 and 7.15. Table 7.6 lists some common grain-storage insects and their optimal growth conditions.

7.1.1.4 Aeration

Grain stored for long periods of time is generally aerated to maintain the overall quality and reduce the risk of storage losses due to insects and mold growth. Aeration is the process of blowing ambient air through grain masses for the purpose of cooling and conditioning grain [12]. It is a well-known and proven integrated pest management (IPM) tool for controlling insects and other risks in stored grain. Aeration reduces or inhibits biological activity by cooling the grain and preventing moisture migration by maintaining a relatively uniform temperature throughout the grain mass [13].

Aeration can cause changes in temperature and moisture content of the stored grain as "aeration fronts" pass through the grain. In bins over 2000 bushels capacity, the grain bulk or mass is so large that it fails to cool uniformly enough to avoid storage problems as outdoor temperatures change with the seasons [14]. The unequal temperature in the grain mass then causes air current to circulate from warm to cold grain. Since warm air holds more moisture than cold, the air moving up through the warm grain center picks up a full load of moisture, depositing some as it moves through the cold grain in the top layer. This causes moisture buildup, molding, and crusting. These minute "convection currents" in the grain cause moisture migration and accumulation that can only be prevented by reducing temperature difference in the grain bulk. The aeration process is illustrated in Figure 7.16.

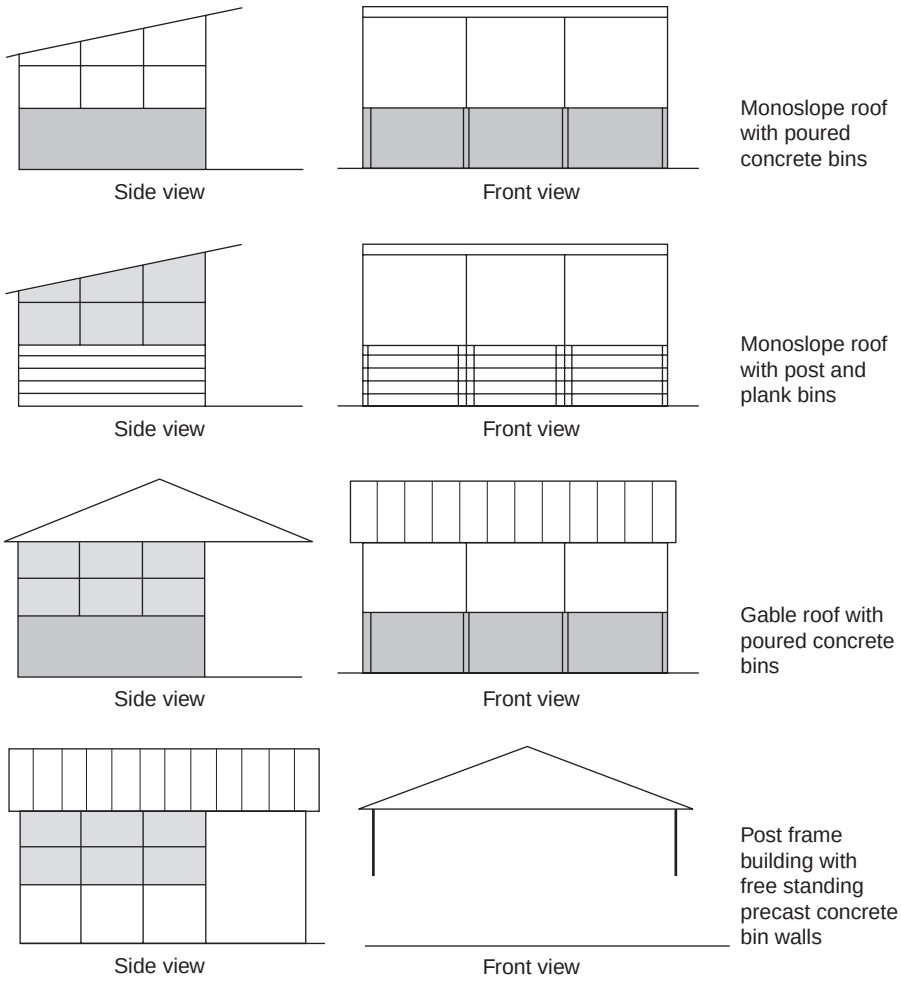

FIGURE 7.6 Typical bulk storage sheds. (From Tyson and Graves [78].)

Typically it is recommended that grain producers maintain the temperature of the stored grain to within +5.5°C (10°F) of the average monthly temperature (depending on location), but not to exceed 15°C (59°F) in the warmer months or less than 0°C (32°F) during the winter [15].

The amount of time required for cooling grain is a function of the airflow rate (fan size), air temperature, and relative humidity of the air. If drying occurs during aeration, evaporative cooling effects will significantly reduce the amount of time required to cool a bin [16]. However, it is not necessary to cool the grain mass below 35 to 40°F because the activity of important storage fungi is very low below these temperatures. The aeration system should not be used to raise the grain temperature above 60°F because mold and insect growth occur at a much faster rate above this temperature [2]. Power requirements for aeration vary with airflow rate, type of grain, and the distance the air must travel through the grain (Table 7.7). The airflow rate should be adequate to cool the entire grain mass before deterioration begins. Normal aeration airflow rates range from 1–2 liters of air per second per cubic meter of grain (1–2 L/s-m^3) (0.08–0.16 cfm/bu). Higher rates should be used (2–6 L/s-m^3) if the grain is stored at higher moisture levels or if a large variance in incoming moisture levels exists [17]. Uniform temperatures through grain mass (Figure 7.17) can be maintained in aerated grain storage if the aeration system has been well designed and is properly operated. Aeration ducts installed in a bin is shown in Figure 7.18. Automatic control of aeration based on ambient temperatures is an inexpensive method to improve the efficiency of aeration systems.

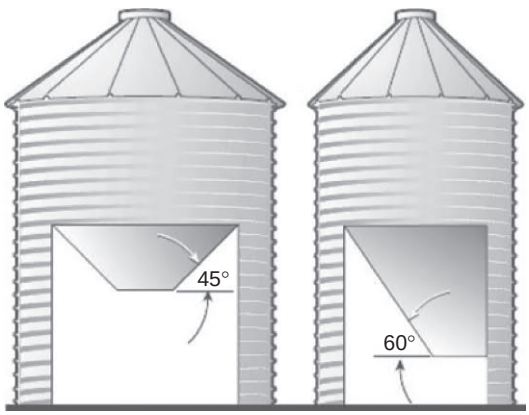

FIGURE 7.7 Center discharge and side discharge bins. (From Harner III and Fairchild [79].)

FIGURE 7.8 Storage bin complex. (From Agriculture and Agri-Food Canada [77].)

FIGURE 7.9 A bin complex. (From Agriculture and Agri-Food Canada [77].)

FIGURE 7.10 Wooden bins. (From Agriculture and Agri-Food Canada [77].)

7.1.1.5 Grain Inspection

The grain surface should be inspected at least every other week throughout the storage period. Signs of hot spots, insect infestations, or other problems that start in the grain mass soon migrate to the surface. Hot spots will be seen as damp,

FIGURE 7.11 Hopper-bottom bins. (From Agriculture and Agri-Food Canada [77].)

FIGURE 7.12 Farm bins. (From Agriculture and Agri-Food Canada [77].)

warm, and musty areas. Insects and mold growth are more likely to show up where broken corn has accumulated.

7.1.1.6 Chemical Methods

Insect infestation in stored grain and grain products can be controlled effectively by fumigation. Over the past 100 years, fumigation has been the most effective method of pest control in stored rice [18]. Up to nine different chemicals have been used as fumigants, but only chlorpyrifos-methyl (Reldan) and phosphine are currently considered safe. Historically, malathion and methyl bromide were used extensively to protect stored rice from insect damage, but many stored-product insect species have developed resistance to malathion [19]. Many of the chemical pesticides used to protect rice and other grains from insect activity are threatened by environmental legislation. For example, methyl bromide depletes the ozone layer and was ordered to be phased out of use by 2005 in developed countries in response to the Montreal Protocol. Other pesticides may be in danger of losing their registration status due to the Food Quality Protection Act and other Environmental Protection Agency rulings [20]. Chlorpyrifos-methyl is the only insecticide labeled for direct application to stored rice, but recently published reports have stated that

TABLE 7.5
Capacity per Unit Depth for Grain Bins of 4.6 m (15 ft) to 14.6 m (48 ft) Diameter

Diameter (ft)	15	18	21	24	27	30	33	36	39	42	48
Diameter (m)	4.6	5.5	6.4	7.3	8.2	9.1	10.1	11.0	11.9	12.8	14.6
Capacity (bu/ft)	141	203	277	362	458	565	684	814	955	1108	1448
Capacity (m^3/m depth)	16.3	23.5	32.0	41.9	53.0	65.3	79.1	94.1	110.4	128.1	167.4

Source: Paulsen and Odekirk [42].

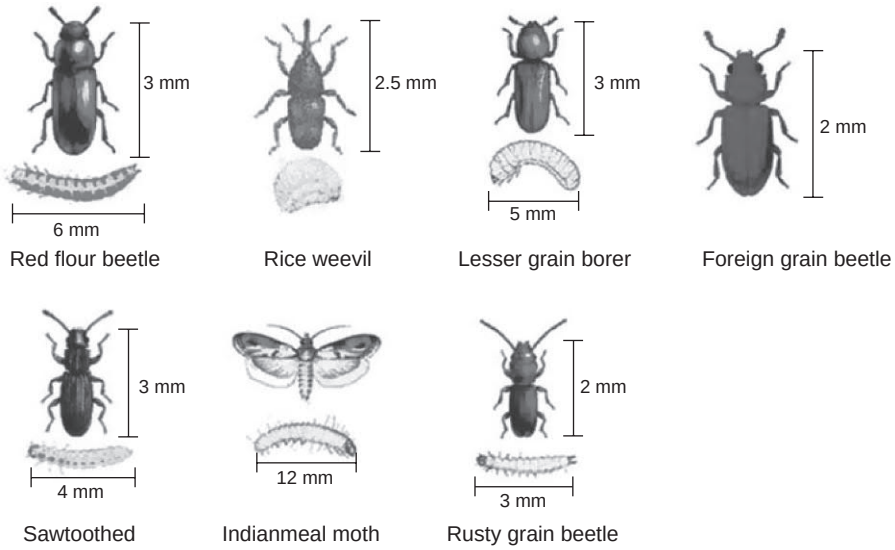

FIGURE 7.13 Some of the grain insects and their dimensions. (From Agriculture and Agri-Food Canada [77].)

lesser grain borers are developing resistance to chlorpyrifos-methyl [21]. Currently, the main chemical option for controlling insects in stored commodities is the fumigant phosphine. The fumigant phosphine has provided an important replacement to methyl bromide in several situations, but phosphine has one important disadvantage: it requires an exposure period of 5 days or longer, which makes it unsuitable for quarantine fumigation [18].

Other possible methods for the protection of stored products from insect infestations would be through the utilization of diatomaceous earth (DE), an inert dust registered to control insects in stored commodities [22], or through the utilization of radiation. Although the newer formulations of DE are more effective than the formulations of the past, they can still affect the physical properties of the stored grain [23]. It may be possible that DE could be an effective method for prevention of infestations, but Arthur [24] demonstrated that extreme conditions, which may not be optimum for grain storage, are needed to allow the DE formulation to be the most effective. Radiation could also supply a direct alternative to the fumigation of rice, but there are few facilities available for this work.

Insects are poikilothermic organisms; therefore, their activity is controlled by their surrounding temperature. The

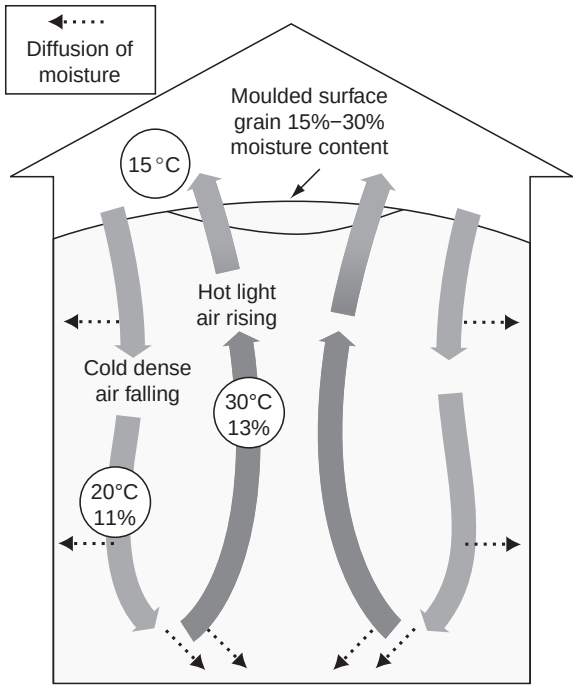

FIGURE 7.14 Air movement in grain. (From Caddick [80].)

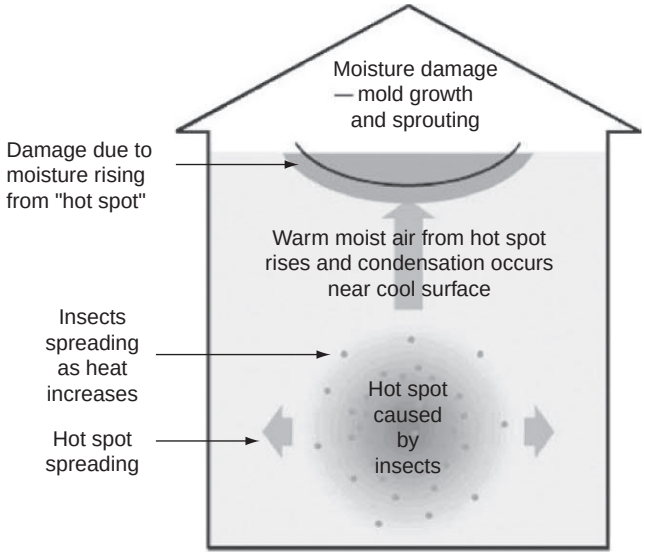

FIGURE 7.15 Development of hotspots due to insect infestation. (From Caddick [80].)

optimal temperatures for their growth and development have been proposed to be between 25°C and 33°C, while 13°C to 25°C and 33°C to 35°C are considered suboptimal [25]. At temperatures below 13°C and above 35°C, most insects will eventually die [26].

7.1.1.7 Rodents

Rodents are among the most important global pests [27]. Three common rodents are the house mouse (*Mus musculus*), the brown rate (*Rattus norvegicus*), and the ship rat (*Rattus rattus*), also known as the roof rat. The brown rat may also be known as the Norway rat, house rat, barn rat, sewer rat, black rat, or wharf rat. Every year, rats in Asia consume food crops that could feed 200 million people for an entire year [28]. Damage due to rodents in Tanzania causes an estimated annual yield loss of 5–15% of corn, corresponding to about $45 million, and food that could feed about 2 million people [29]. In parts of South America, native rodents cause crop damage varying between 5 and 40% of total production [30]. It is estimated that one rat can consume about 10 g of feed per day and destroy ten times this amount during feeding with its droppings and urine [31]. Infestation of feed grains by mites can seriously reduce the nutritional quality the feed grains and acceptance by animals [32]. There is also the possibility of mites causing allergy problems in workers handling contaminated grain and grain products [33].

Rodent killing as a single control measure is expensive and effective for only brief periods of time. It is most effective if carried out during the winter when reproduction is at its lowest level. Two forms of rodent control are normally practiced. Trapping is the most common method of rat killing. Traps may or may not be baited but should always be placed in areas of rodent activity. Bacon, peanut butter, bread, and nutmeats make suitable baits. Mousetraps should be placed at intervals of about 1 m (3 to 4 feet); rat traps should be set 4.5 to 9 m (15 to 30 feet) apart. Traps are an alternative to rodenticides, especially where chemicals cannot be used; however, the use of traps is more labor-intensive than chemicals.

TABLE 7.6
Optimal Development Conditions of Some Common Insect Species Found in Grain Storages

Insect	Temperature (°C)	Relative Humidity (%)
Angoumois grain moth (*Sitotroga cerealella*)	26–30	75
Indian meal moth (*Plodia interpunctella*)	26–29	70
Khapra beetle (*Trogoderma granarium*)	33–37	25
Larger grain borer (*Prostephanus truncates*)	25–32	80
Lesser grain borer (*Rhyzopertha dominica*)	32–34	50–60
Red flour beetle (*Tribolium castaneum*)	32–35	70–75
Rice weevil (*Sitophilus oryzae*)	26–31	70
Rusty flour beetle (*Cryptolestes ferrugineus*)	33	70–80
Sawtoothed grain beetle (*Oryzaephilus surinamensis*)	31–34	90
Maize weevil (*Sitophilus oryzae*)	26–31	70

Source: Montross et al. [9].

7.1.2 Structural Considerations: Warehouse and Silo

7.1.2.1 Warehouses

Warehouses are used to store milled rice. Millers normally use bags to store rice, which may vary in size from 50 lb (22.7 kg) to 1 tonne. Warehouses are much less efficient than bins and higher costs associated with storage but are very useful for higher-valued products such as milled and specialty rice [34].

7.1.2.2 Grain Bulk

The design of bins and silos involve bulk materials and geometric and structural considerations. The frictional and cohesive properties of bulk grains vary from one grain to another. In addition, a given bulk grain's flow properties can vary dramatically with changes in numerous parameters, such as particle size, moisture, temperature, and consolidating pressure. Some of the design techniques that are commonly used take into account grain properties such as specific weight (Υ), angle of internal friction (ϕ), and grain-to-wall friction coefficient.

7.1.2.3 Storage Structure Design

While considering the geometric design of a silo, potential problems include arching across an outlet, rat-holing (Figure 7.19) through the material, and the flow pattern during discharge [35]. The arching or rat-holing is primarily related to the grain's cohesiveness, while its flow pattern during discharge depends upon internal friction as well as the friction that

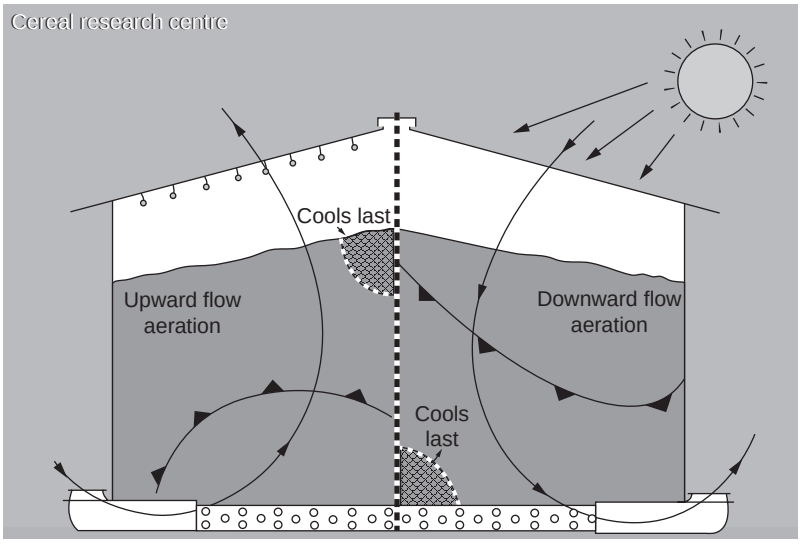

FIGURE 7.16 Aeration process illustrations. (From Agriculture and Agri-Food Canada [77].)

develops between the grains and the hopper walls of the silo. The goal of geometric design is to maximize the usable capacity of a silo while minimizing its capital cost, overall height, etc. [35].

Silo design procedures include the selection of the optimum hopper angles and minimum outlet dimensions. The ideal discharge mode is one where, at steady state, all material flows. The structural design of a silo requires knowledge of the distribution of pressures and shear stresses on its walls (caused by the stored grain) and how that distribution varies during charging, storage at rest, discharging, and recharging [35].

Grain storage bins are generally designed as thin-walled cylindrical shells and typically loaded and unloaded along the line of their central axis. The loads exerted by grain on silo structures can be grouped into two categories: those because of initial fill and those due to flow. When grain is poured into a bin, it forms an angle from the horizontal, called the angle of repose. The outflow hopper at the bottom of a bin must be cone-shaped and have a slope greater than the angle of repose, or the grain will not flow out. Smaller bins require a steeper slope because of the greater friction on the sides of the hopper. The angle of repose of some cereal grains is presented in Table 7.8. The lateral pressure of the grain on bin walls is about 0.3 to 0.6 of the vertical pressure, and the vertical pressure increases very little after a depth of about three times the bin diameter [7]. Grain settles or packs during storage. Lightweight grain such as oats may pack to lose as much as 28% of its volume.

Design values for the coefficient of friction of grains on various materials are important. However, the coefficient of friction varies not only with each type of grain but also with experimental conditions such as moisture content. Hence, the selection of the coefficient of friction will depend on the experience of the individual engineer [36]. Design parameters for some of the grains at different moisture contents are presented in Table 7.9.

Several countries have adopted codes and standards for bin designs to ensure safe and better quality structures. They include the Canadian Farm Building Code [37] and EP433 [38]. In EP433, a maximum of 834 kg/m^3 is recommended for the bulk density of any free-flowing grain, and other material properties depend only on wall material [36]. The properties of some of the wall materials are presented in Table 7.10.

ASAE Standards [38] give design recommendations for axial symmetric states of stress. However, during the

TABLE 7.7
Approximate Static Pressure

Airflow		Static Pressure (Inches of Water)	
Rate (cfm/bu)*	Distance through Grain, ft	Maize	Wheat
1/20	50	1.0	3.3
1/10	50	1.8	6.9
1/20	100	3.0	13.0
1/10	100	7.3	25.0

Source: Bailey [10].
* ft^3/min/bushel (≈ m^3/min/ton)

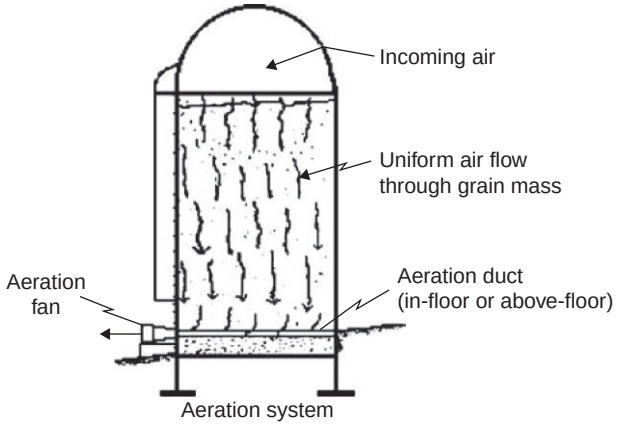

FIGURE 7.17 Aeration system. (From Ministry of Agriculture [81].)

FIGURE 7.18 Aeration ducts in a bin. (From Agriculture and Agri-Food Canada [77].)

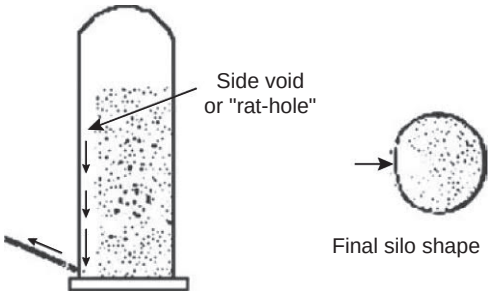

FIGURE 7.19 Rat-holing leads to unbalanced sidewall forces. (From Ministry of Agriculture [81].)

TABLE 7.8
Angle of Repose of Selected Cereal Grains

Grain	Bulk Density (kg/m³)	Kernel Density (kg/m³)	Porosity (%)	Angle of Repose, Filling	Emptying
Barley (Bedford)	664	1346	44	24	26
Oats (Fidler)	555	1315	52	27	25
Wheat, bread (Columbus)	780	1379	38	26	23
Rye (Gazelle)	760	1406	41	25	21
Wheat, durum (Coulter)	744	1377	41	23	21

Sources: Muir and Sinha [88]; Rameshbaba et al. [89].

operation of a grain facility, certain loading and/or unloading conditions can create a nonuniform distribution of pressure within a grain bin. The highest asymmetry of bin load is thought to occur during eccentric unloading. Nonsymmetrical bin loads, which occur during eccentric discharge, are considered to be a major cause of bin failure [39].

The design of grain silos requires the designer to understand not only the principles of structural design but also the properties of stored grains. Eurocode for bin design has been published to provide guidelines for silo designers [40]. This design standard predicts bin loads using Janssen's equation.

Recently, numerical methods have been used in grain silo design. It has been possible to better model the behavior of grain inside a bin. However, in order to use these methods, it is necessary to consider additional grain properties, such as elastic modulus (E), Poisson's ratio (ν), and dilatancy angle (ψ). However, very limited information is available on these design parameters [41]. Tables 7.11 and 7.12 present some additional grain properties that can be used in numerical methods of silo design. Storage capacities of grain bins are shown in Table 7.13.

TABLE 7.9
Design Parameters for Selected Grains

Grain	MC (%)	WM	μ	K	W (kg/m³)
Barley	11.0	Smooth steel	0.10	0.4	620
Barley	11.0	Corrugated steel	0.35	0.6	620
Barley	11.0	Plywood	0.30	0.6	620
Barley	11.0	Concrete	0.35	0.6	620
Corn shelled	11.0	Smooth steel	0.20	0.4	720
Corn shelled	11.0	Corrugated steel	0.35	0.6	720
Corn shelled	11.0	Plywood	0.30	0.6	720
Corn shelled	1.0	Concrete	0.35	0.6	720
Wheat	11.0	Smooth steel	0.10	0.4	770
Wheat	11.0	Corrugated steel	0.35	0.6	770
Wheat	11.0	Plywood	0.30	0.6	770
Wheat	11.0	Concrete	0.35	0.6	770

Source: Ni [36].
WM = Wall material, μ = Coefficient of friction between the grain and bin wall, K = Ratio of lateral to vertical pressure, W = Grain bulk density.

TABLE 7.10
Material Properties for EP433

Wall Material	μ	K
Concrete	0.40	0.5
Corrugated steel	0.37	0.5
Smooth steel	0.30	0.5

Source: Ni [36].

7.2 GRAIN HANDLING

7.2.1 Conveyors

There are various types of grain conveyors, such as en-masse conveyor, U-trough conveyors, and tube augers.

7.2.1.1 En-Masse and Shrouded Conveyors

En-masse conveyors (Figure 7.20) can be designed to move grain in any application, at any operating angle. However, an en-masse conveyor starts to lose capacity with an operating

TABLE 7.11
Recommended Values of the Angle of Internal Friction (ϕ), Apparent Cohesion ©, Dilatancy Angle (ψ), and Poisson's Ratio (ν) for Selected Grains at Certain Moisture Content

Grain	ϕ (°)	C (kPa)	Ψ	ν	MC (%)
Barley (krona)	24.8–26.6	0.58–5.19	7.0–18.9	0.33–0.35	12.80
Barley (Kym)	21.6–25.4	0–10.72	4.0–4.9	0.35–0.36	11.92
Chickpea (Eulalia)	26.8–28.8	0–8.25	27.1–34.4	0.26–0.27	10.51
Wheat (Camacho)	20.8–24.6	2.87–13.16	6.0–14.4	0.27–0.37	11.15

Source: Moya et al. [41].

TABLE 7.12
Recommended Values for Real Specific Weight (Υ_r), Apparent Specific Weight (Υ_{ap}), and Modulus of Elasticity (E) at Certain Moisture Content

Grain	Υ_r (N/m^3)	Υ_{ap} (N/m^3)	*E* (kPa)	MC (%)
Barley (Krona)	11707	6451	1267–1372	12.80
Chickpea (Eulalia)	13218	8313	5780–5903	10.51
Oats (Prevision)	10225	4747	413–571	10.00
Wheat (Horzal)	12575	8147	5048–5211	11.03

Source: Moya et al. [41].

angle greater than horizontal, and the maximum operating angle is often considered to be 7° [42]. En-masse conveyors do less damage to grain than augers and are often recommended in high-capacity handling situations because of lower power requirements. Once the operating angle exceeds 7°, it is recommended to use a shrouded conveyor. A shrouded conveyor acts more like a drag conveyor, pulling grain along the inside of an enclosed chamber. Shrouded conveyors are typically used with operating angles up to 45° [42].

7.2.1.2 U-Troughs and Tube Augers

U-trough conveyors are often used for horizontal applications for reclaim from bins or across the roofs of bins. Tube augers and U-troughs will both do less damage to grain if run full and at low speeds. Both types of augers lose about 40% of their capacity in wet grain [42]. The capacities and power requirements for horizontal screw conveyors (augers) are presented in Tables 7.14 and 7.15, respectively. The auger capacities will vary with grain type, condition, and loading method. The information presented here is to be used as only a guide and the power requirement will vary with condition and type of grain, loading factor, revolutions per minute (rpm), use of reduction pulleys, and speed reducers. For high moisture grain, the power should be multiplied by a factor of 1.5.

7.2.1.3 Pneumatic Conveyors

Pneumatic conveyors are used for transporting dry grain away from grain dryers to storage bins. Some advantages of pneumatic conveyors are they are self-cleaning, allowing for transport of different products without contamination; dust is totally enclosed, except at discharge; and safe (no moving parts other than blower and airlock). Their disadvantages [42]: power requirements greater than other methods; noisy to operate; grain damage can occur if the system is not operated properly, so grain velocities remain around 762 to 914 m/min (2500–3000 ft/m). Typical pneumatic-conveyor characteristics are presented in Table 7.16.

7.2.1.4 Bucket Elevators

Bucket elevators provide an effective way to distribute and automate handling of grain. Spouts normally come in diameters of 102 mm (4 in.), 152 mm (6 in.), 203 mm (8 in.), 254 mm (10 in.), 305 mm (12 in.), or 356 mm (14 in.) [42]. The power requirement of a bucket elevator depends on the capacity and product-elevating height (Table 7.17).

7.2.2 Controlled Atmospheric Storage of Grain

Controlled atmospheric (CA) storage of grains includes commodity-modified CA storage and artificially modified CA storage [43]. In the case of commodity-modified storage, respiration of the grain and the microorganisms reduce the O_2 and increases the CO_2. The atmosphere in a modified storage is changed by injecting N_2 or CO_2 into the system [9]. Nitrogen-producing exothermic generators are available commercially for altering the intragranular gas composition in a grain storage system. Carbon dioxide (CO_2) is another gas, which can also be used for CA storage of grains.

7.3 MILLING

7.3.1 Grain Milling Operations

The term *milling* refers to the size reduction of granular material, but for cereal grains, the term has different connotations.

TABLE 7.13
Storage Capacity of Bins [36]

Bin Diameter (m)	Bin Height (m)	Stored Capability (Ton)
4.0	3.0	38
4.0	4.0	48
4.0	5.0	58
4.0	6.0	68
4.0	7.0	79
4.0	8.0	89
4.0	9.0	99
4.0	10.0	109
5.0	3.0	61
5.0	4.0	77
5.0	5.0	93
5.0	6.0	110
5.0	7.0	126
5.0	8.0	142
5.0	9.0	158
5.0	10	174
6.0	4.0	115
6.0	5.0	138
6.0	6.0	162
6.0	7.0	185
6.0	8.0	208
6.0	9.0	237
6.0	10	254
7.0	4.0	162
7.0	5.0	194
7.0	6.0	225
7.0	7.0	256
7.0	8.0	288
7.0	9.0	319
7.0	10	351
8.0	4.0	218
8.0	5.0	260
8.0	6.0	301
8.0	7.0	342
8.0	8.0	383
8.0	9.0	424
8.0	10	465

Wheat milling means wheat grinding to prepare flour. Rice milling includes operations like dehusking and polishing; whereas pulse milling may involve processing operations such as husk separation, splitting of kernels, and polishing, or just polishing. The dry milling of cereals consists of grain cleaning, tempering and conditioning, and roller milling. It is primarily concerned with separation of the anatomical parts of grain. Roller mills are considered to be the workhorses of the grain milling industry. In flour milling, roller mills perform bran separation as well as size reduction. First-break, or the first roller mill operation in the milling process, performs the first bran separation by opening the wheat kernels with minimum bran breakage. Bran coming out in the form of flakes ensures ease of separation from the endosperm in succeeding stages. Mechanical energy is required to impart compressive and shear forces that break wheat kernels and reduce the size of endosperm particles [44].

FIGURE 7.20 En-masse conveyor. (From GSI Grain Systems [82].)

7.3.2 Specialty Milling

Identity-preserved (IP) grains are referred to as specialty, high value, premium, or niche market grains. They are produced with a specific end-use in mind, perhaps human food, a specific kind of animal feed, cosmetics, pharmaceuticals, or industrial use [45]. An example of an identity-preserved cereal grain is corn: high oil, endosperm/food grade, white, high amylose, waxy, and nutritionally dense (low phytase, high lysine or methionine).

Corn wet milling is a complicated, large-scale, and efficient industrial process designed to separate the chemical components from corn kernels. The success of wet milling, in terms of maximum yields, is largely dependent on the success of the steeping process. Improper steeping, or steeping of corn kernels that have unusual physical or chemical structures, results in lost product and lower profits. Steeping processes, however, are still based largely on an art that was developed more than 100 years ago [46]. As the market increases for specialty corn, corn genetically bred with unique starch characteristics, or corn with altered chemical composition, a thorough scientific understanding of steeping chemistry and the entire wet milling process will become increasingly important.

The wet milling process goes a step further than cleaning, by separating some of those anatomical parts into their chemical constituents, such as starch, protein, oil, and fiber, instead of bran, germ, and endosperm [7]. The steps involved in corn wet milling is shown in Figure 7.21. The different components derived from the corn wet milling operation are shown in Figure 7.22.

The effectiveness of the dry-grind corn process (Figure 7.23) lies in the complete conversion of starch into ethanol. It is important that starch be available for digesting enzymes

TABLE 7.14
Capacities for Horizontal Screw Conveyors with Clean Dry Corn at 90% Loading (Which Is Usually Maximum)

Auger Diameter, mm (in.)	Auger, rpm	Capacity, t/h (bph) per 100 rpm	Capacity, t/h (bph) at Stated rpm	% Loss from Horizontal for 45° Angle of Operation	% Loss from Horizontal for 90° Angle of Operation	% Loss for 25% Moisture Content Corn
102 (4)	312	1.5 (60)	4.7 (187)	20	50	40
102 (4)	875	1.5 (60)	13.3 (525)	20	50	40
152 (6)	276	6.4 (250)	17.5 (690)	20	50	40
152 (6)	510	6.4 (250)	32.4 (1275)	20	50	40
203 (8)	269	12.7 (500)	34.2 (1345)	20	50	40
203 (8)	438	12.7 (500) (1200)	55.7 (2190)	20	50	40
254 (10)	261	30.1 (1200)	79.6 (3132)	20	50	40
254 (10)	350	30.1 (1200) (2000)	106.8 (4200)	20	50	40
305 (12)	285	50.8 (2000)	144.9 (5700)	20	50	40
305 (12)	350	50.8 (2000)	178.0 (7000)	20	50	40
356 (14)	80	101.7 (4000)	81.4 (3200)	20	50	40
356 (14)	200	101.7 (4000)	203.4 (8000)	20	50	40

Source: Hutchinson Manufacturing [90].

TABLE 7.15
Length of Horizontal Auger That Can Be Powered by a Given Motor Horsepower

Motor (kW (hp))	Length of Auger (m (ft))			
	152 mm (6 in.) diameter	203 mm (8 in.) diameter	254 mm (10 in.) diameter	305 mm (12 in.) diameter
1.1 (1.5)	4.6 (15)	—	—	—
1.5 (2)	7.6 (25)	4.6 (15)	3.0 (10)	—
2.2 (3)	13.7 (45)	7.6 (25)	6.1 (20)	4.6 (15)
3.4 (5)	21.3 (70)	15.2 (50)	10.7 (35)	7.6 (25)
5.6 (7.5)	33.5 (110)	24.4 (80)	15.2 (50)	12.2 (40)
7.5 (10)	—	33.5 (110)	24.4 (80)	15.2 (50)
11.2 (15)	—	—	33.5 (110)	24.4 (80)
Capacity with corn, t/h (bph)	22.9–0.5 (900–1200)	38.1–50.8 (1500–2000)	63.6–89.0 (2500–3500)	101.7–127.1 (4000–5000)

Source: Hutchinson Manufacturing [90].

such as a-amylase and glucoamylase. This is possible only if starch granules are exposed during grinding and cooking processes. It has been observed that the starch granule structure differs significantly in hard and soft endosperms. The protein and starch matrix also is different in endosperm fractions. It has been observed that hard and soft endosperm in corn significantly differs in their dry milling characteristics, and these differences may be due to structural differences in the protein–starch matrix and nature of endosperm starch granules. Endosperm hardness might have an effect on starch conversion into glucose and the fermentability of glucose into ethanol [47]. Optimization of the dry and wet milling processes require timely knowledge of the distribution of milled products and their by-products. Although the machinery used in various milling operations is important, control and operation of the machines by the operator is even more critical.

7.3.3 Rice Milling and Processing

An ideal rice mill removes the bran layers and germ from brown rice kernels with minimal kernel breakage and preserves each kernel in its original shape [48]. The quality of milled rice is largely determined by the yield of well-milled, whole kernels, referred to in the rice industry as head rice [49]. Broken rice kernels are sold at a much lower price than head rice.

The most common huller used in milling operations is a rubber-roll sheller (Figure 7.24). They are preferred because of their efficiency in removing hull (>90%). In a modern rice mill, a pair of rubber-lined rollers are mounted in an enclosed chamber and driven at a friction ratio. A typical unit using 254 mm nominal width × 254 mm outer diameter rollers run at 1.28:1 friction ratio with the faster roller at 900 rpm. The nip of the rollers is manually adjusted during

TABLE 7.16
Typical Pneumatic-Conveyer Characteristics

Pipe Diameter (cm)	Capacity (m^3/h)	Power (kW)
7.6	14.1	7.6
10.2	24.7	11.5
12.7	42.3	15.3–22.9
15.2	70.5	30.6–38.2

Source: Midwest Plan Service [91].

TABLE 7.17
Typical Bucket Elevator Data for Maize

Capacity (m^3/h)	Belt Speed (m/min)	Bucket Size (cm)	Bucket Spacing (cm)	Power (kW/m)
35	101	22.9 × 12.7	30.5	0.098
53	115	22.9 × 12.7	22.9	0.147
70	101	22.9 × 12.7	15.2	0.196
8	129	22.9 × 12.7	15.2	0.245
106	151	22.9 × 12.7	15.2	0.294
141	151	22.9 × 15.2	16.5	0.392

Source: Brooker et al. [92].

dehusking to compensate for the gradual abrasion of the rubber surface and its thermal softening. The paddy passing through the rollers nip is subjected to both compressive and shearing forces. When these forces are correctly predetermined for a particular grade of paddy (by rubber hardness, resilience, rollers nip, speed, and friction ratio), the paddy will remain substantially intact after going through, while the husk is broken off. In practice, dehusking efficiency can be as high as 90% per pass. The products from a rice mill are head rice, brokens, rice bran, rice polish, and the hulls. In general, paddy rice yields 20% hulls and about 2% polish [7]. A schematic diagram of rice milling operation is presented in Figure 7.25.

The pearler or milling machine is the most critical machine in a rice mill, in which barn is removed and also in which most of the breakage occurs [46]. Figure 7.26 shows a mill-top one-pass rice pearler combined with a husker. The degree of milling can be controlled by varying the pressure and thereby regulating the average residence time in the chamber. Figure 7.27 shows a paddy husker.

Parboiling of rice is considered as a means of reducing broken losses during milling. The idea is based on the attributes of the apparent hardness of parboiled rice. Breakage of nonparboiled rice during milling is generally related to various physical properties of the grain and environmental conditions during the growing season, harvesting, handling, and storage of rice [50]. Rice grains with stress fissures break more readily than sound kernels during handling, milling, and transportation, thereby reducing the quality and market value of the grain [51]. The value of the broken rice grain is often one-half the value of the whole grain [52]. The major source of broken rice during milling is fissured grains [53].

7.3.4 Dehulling and Splitting of Pulses

In many countries of the world, pulses are initially processed by removing the hull and splitting into dicotylednous

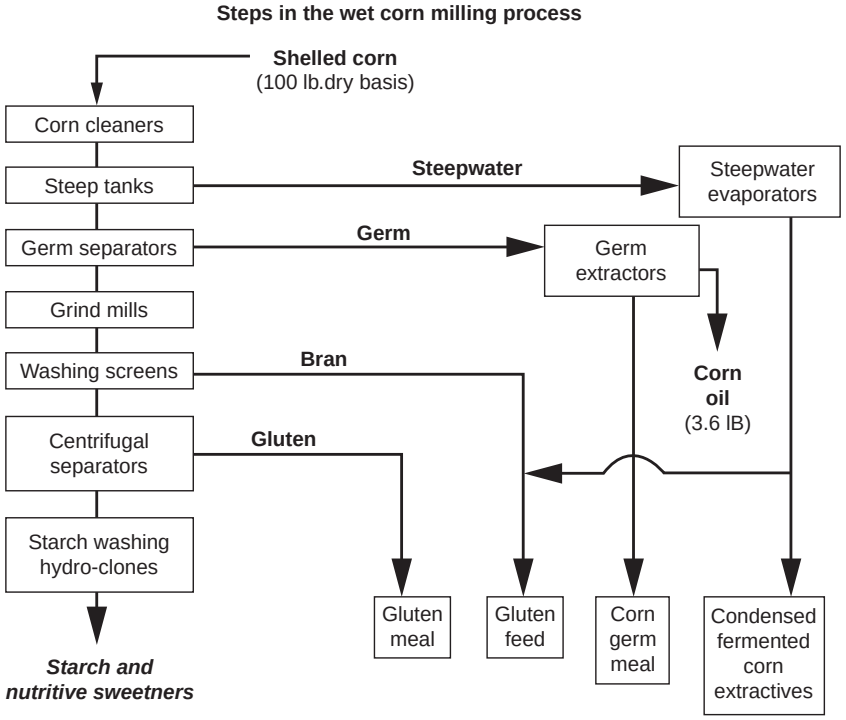

FIGURE 7.21 Overview of a wet corn milling process. (From Schroeder [83].)

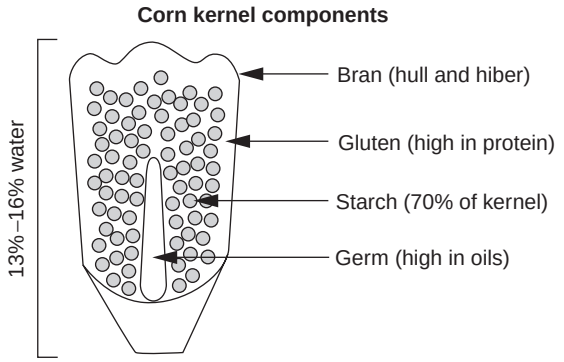

FIGURE 7.22 Component derived from corn in the wet milling process. (From Schroeder [83].)

components. The dehulling operation is usually performed in two steps: the first involves loosening the husk from the cotyledons, and the second removing the husk from cotyledons and splitting them using a roller machine or stone chakki.

Graded grains are made to pass through a roller machine, which causes a mild abrasion (tempering operation). The tempering operation causes slight scratches on the seeds and enhances their oil and water-absorbing efficiency leading to the loosening of the testar. The grain is then treated with oil and water, and then spread on drying yards to dry under the sun. For dehulling of conditioned pulses, emery rollers, called gota machines, are used. In one pass or single operation, about 50% of pulses are dehusked. Dehusked pulses are split into two parts. Dehusked split pulses are separated by sieving and the husk is aspirated off. Unsplit dehusked pulses and tail pulses are again dehusked and milled in a similar way. The whole process is repeated two or three times until all of the pulses are dehusked and split off.

7.3.4.1 Wet Milling of Pulses

There are two types of conventional pulse milling methods in India: wet milling and dry milling. A flow diagram of wet milling is given in Figure 7.28.

7.3.4.2 Dry Milling of Pulses

Hulling of legumes on a commercial scale is generally based on dry-processing techniques. Many of the operations, particularly husking and splitting, are mechanized. For all types of pulses, there is no common processing method. However, some general operations of the dry milling method such as cleaning and grading, rolling or splitting, soiling and moistening, and drying and milling are considered (Figure 7.29). Removal of the loosened husks from the pulses in the dry milling technique is done in small machines. Hand- or power-operated underrunner disc shellers or grinders with emery or stone contact surfaces are normally used. A plate mill with a blunt contact surface is sometimes used both to husk and split soaked and dried pulses. After aspirating or winnowing off the husk, the split cotyledons are separated by sieving. Remaining unsplit whole grains are similarly processed until almost all the grain is husked. In certain parts of India, oil-treated and sun-dried grains are husked in an Engelberg-type rice huller after being mixed with 2–3% stone powder. Sound kernels are removed by sieving, while the husk, powder, and small brokers remain in the stone powder [54]. Figure 7.30 shows the facility for dehulling and splitting of a variety of pulses at the Canadian International Grains Institute [55].

7.3.5 Milling of Pulses

Dry whole seeds of pulses possess a fibrous seed coat or testa (husk, hull, or skin). The seed coat is often indigestible; therefore, pulses are mainly consumed after dehusking to improve their palatability and taste. In most parts of the

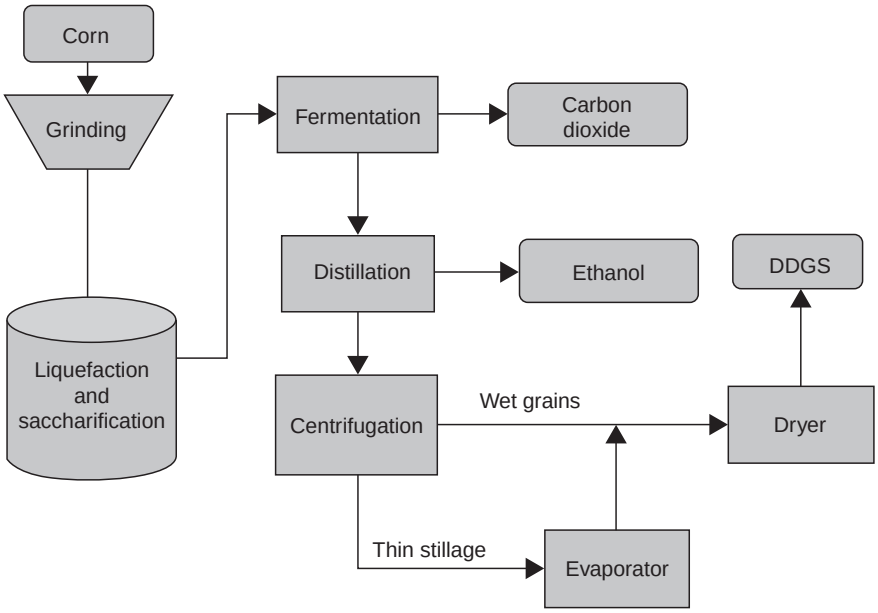

FIGURE 7.23 Dry grind corn process. (From Murthy et al. [47].)

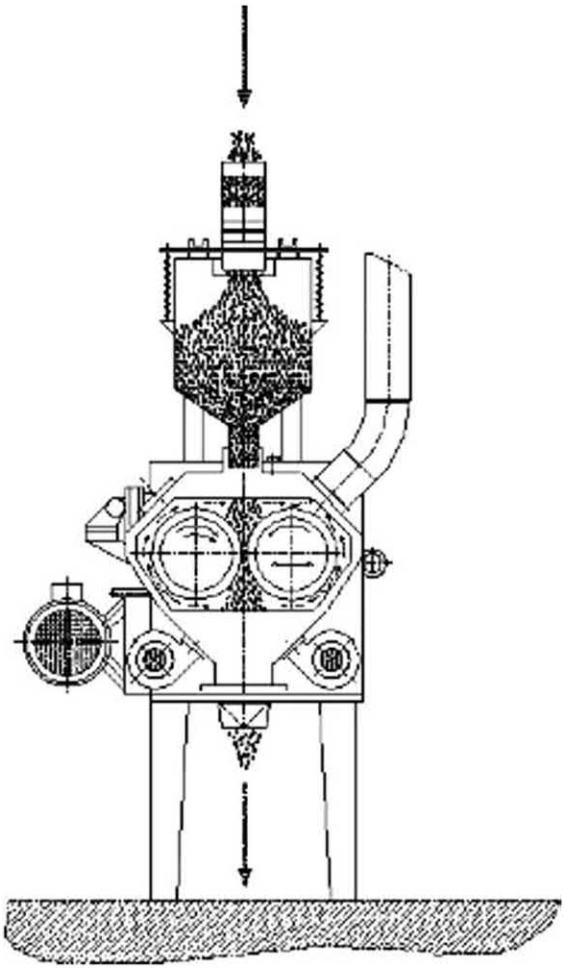

FIGURE 7.24 Rubber-roll sheller. (From Schule [84].)

world, pulses are traditionally consumed either in the whole or in the form of dehusked split pulses. Dehulling, therefore, is an important primary processing activity. Milling provides dehulled cotyledons with better appearance, texture, and cooking qualities [56].

7.3.5.1 Milling Technologies

Milling of pulses involves the removal of the outer husk and splitting the grain into two equal halves. Generally, the husk is much more tightly held by the kernel of some pulses than most cereals, causing dehusking of pulses to be a problem. The method of alternate wetting and drying is used to facilitate dehusking and splitting of pulses. In developing countries, the dehusked pulses are produced by traditional milling methods. In traditional milling, loosening of the husk by conditioning is not sufficient. Therefore, a large amount of abrasive force is applied for the complete dehusking of the grains, which results in high losses in the form of brokens and powder. Consequently, the yield of split pulses in traditional mills is only 65–70% in comparison to 82–85% potential yield [57].

Pulse milling constitutes two major steps: loosening of the husk, followed by removal of the loosened husk by suitable milling machinery. The first step is referred to as premilling, whereas the second is referred to as milling or dehulling. Loosening of the husk is achieved either by a wet or dry method. In the wet method, the grains are soaked in water for a few hours, drained, left in heaps (usually overnight), and dried in the sun. In the dry method, cleaned and size-graded grains are mixed with a small amount of oil, usually after scarification of husk. This scarification of husk is commonly called pitting and is done to facilitate the oil penetration between the husk and the cotyledons. Oil-treated grains are heaped overnight and then dried in the sun for 2–5 days, with intermittent water spraying and mixing. In both of the premilling treatments, adherence of the husk to the cotyledon weakens and, as a result, its removal becomes easy [56].

There is no common processing method for all types of pulses. However, some general operations of dry milling have been described here. General operations of dry milling include cleaning and grading, rolling or pitting, oiling, moistening, drying, and milling [58]. In India, traditional Dhal milling is the most common processing method used for most pulses.

7.3.5.2 Unit Operations

Conventional pulse milling involves many unit operations, such as cleaning the raw material, size-grading, scarification of the husk (pitting), oil mixing, water mixing, drying, dehulling, splitting, aspiration of husk separation of brokens and splits, and, finally, polishing [56].

7.3.5.2.1 Cleaning and Grading

The basic step involved in the processing of pulse products is the cleaning of the raw material. In cleaning, the main aim is to remove any foreign material, adhering soils, dust, chaff, and fungal spores that are attached to or mixed with the product. A simple cleaning unit may consist of a vibratory inclined sieve, hopper, grain collector, waste collector, and motor. The vibratory sieves have different size openings to match the requirements of the type of final product being processed. Grains can be graded according to size by hand-operated or power-operated cleaners and graders.

Cleaning facilities also usually grade the pulse seeds, primarily based on size, color, and absence of visible damage or infection [59]. Color and size are important grading factors for certain pulses. The Canadian Grain Commission (CGC) has developed a grading instrument for assessing the color and size distribution of lentils. Modules for grading other pulses, such as peas, chickpeas, and beans are under development [60].

7.3.5.2.2 Color Sorting

Color sorting is another means of adding value to pulse seeds. The technology uses ultraviolet light to differentiate seed coat color as seeds pass through the machine. However, this advanced technology is very expensive and only a very few pulse processors in some developed countries can possess it.

7.3.5.2.3 Pitting

An emery roller machine is used to achieve cracking and scratching of clean pulses passing through it. This leads to the facilitating of water absorption. The clearance between

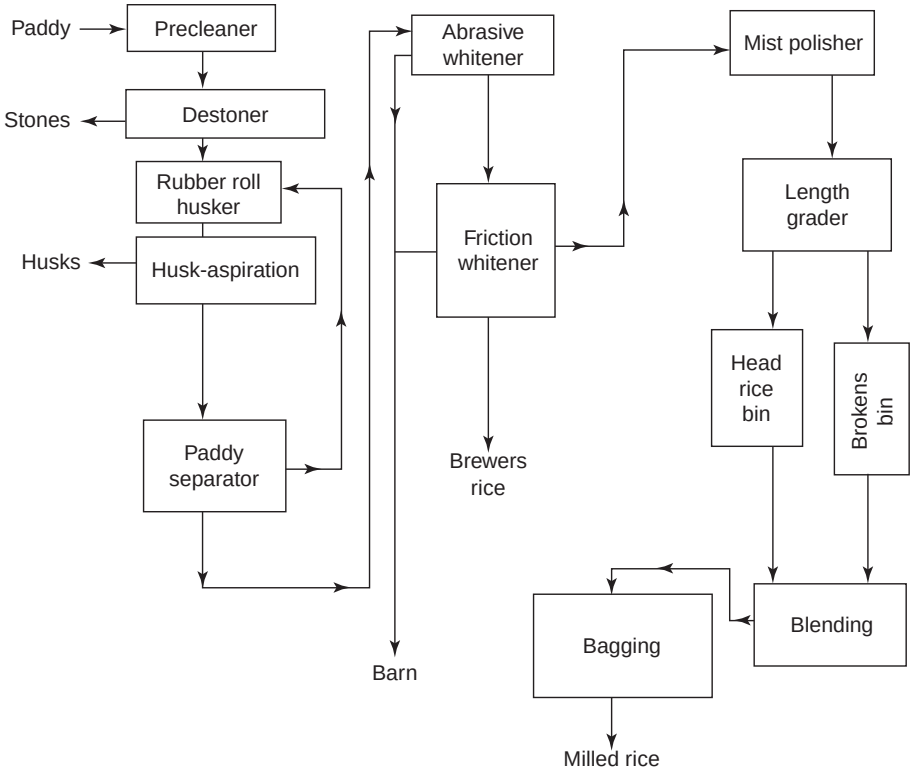

FIGURE 7.25 Schematic representation of rice milling operation.

the emery roller and cage (housing) gradually narrows from inlet to outlet. Cracking and scratching of husks take place mainly by friction between pulses and emery as the material is passed through the narrowing clearance. Some of the pulses are dehusked and split during this operation and are then separated by sieving. Pitting or scratching of grain legumes is done using a roller machine.

7.3.5.2.4 Pretreatment with Oil

A screw conveyor allows passing the scratched or pitted material through it and mixing of some edible oil, such as linseed oil (1.5 to 2.5 kg/tonne of pulses). Then they are placed on floors for about 12 h to diffuse the oil.

7.3.5.2.5 Conditioning

Pulses are conditioned by alternate wetting and drying. Moisture (3–5%) is added to the pulses after sun drying for a certain period and then tempering is done for about 8 h. The grain is dried in the sun again. By allowing water to drop from an overhead tank on the pulses that are passed through a screw conveyor, the addition of moisture to the pulses is achieved. The screw is slowly rotated (50–70 rpm) to achieve proper mixing of oil–water with the grain. The length and width of the conveyors range between 1500–2500 mm and 200–300 mm, respectively [56]. Until all pulses are sufficiently conditioned, the whole process of alternate wetting and drying is continued for 2 to 4 days. Pulses are finally dried to about 10–12% moisture content. Mechanical hot-air-drying systems can also be used for drying of pulses.

7.3.5.2.6 Dehusking and Splitting

Dehusking is a process that reduces the fiber content and improves the appearance, texture, cooking quality, palatability, and digestibility of grains [61]. In one pass or single operation, about 50% of pulses are dehusked. Dehusked pulses are split into two parts. The split pulses are then separated by sieving and the husk is aspirated off. Unsplit dehusked pulses and tail pulses are again dehusked and milled in a similar way. Until the remaining pulses are dehusked and split, the whole process is repeated two or three times. Teckchandani and Mukherjee [62] reported that recovery of dehusked splits is in the range of 68–75% in three passes. A simple box-type aspirator with a suction fan or a cyclone-type separating system can be used. Split pulses are often used by processors in stews and soups because they cook much faster than whole pulses [59].

For splitting, various machines similar to underrunner disc shellers, attrition mills, roller machines or impact machines are used. In India, carborundum emery rollers are used for dehusking, while burr grinders are used for splitting [63]. Dehusking and splitting can also be achieved in a roller machine and can be done as either simultaneous or separate operations. In separate operations, the water-treated and sun-dried seeds are split in sheller machines [64].

7.3.5.2.7 Improved Dehusking Method

A hot air stream of 300°C is passed through the grains to reach a critical point, which makes the seed coat brittle. Then the seeds are allowed to pass through the pulse-dehulling

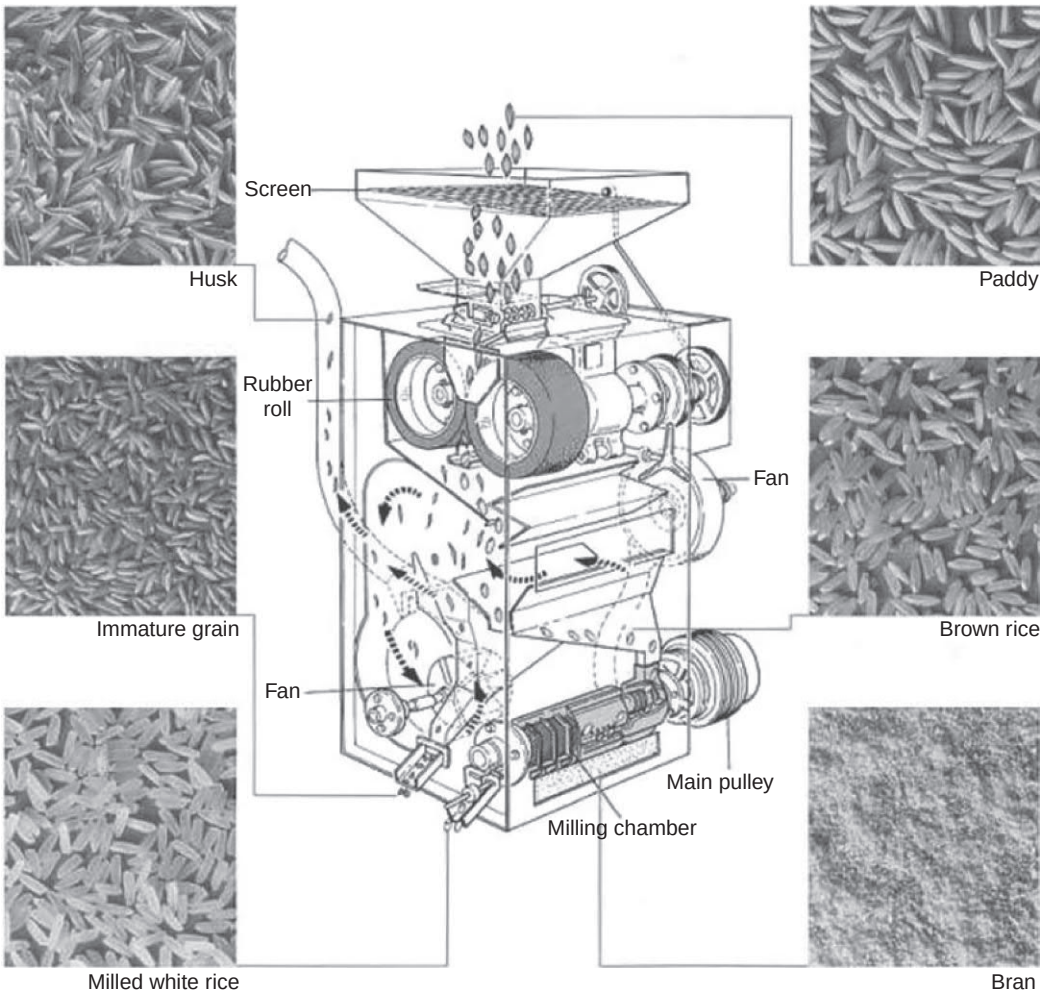

FIGURE 7.26 One-pass rice pearler combined with husker. (From Stake Corporation [85].)

machine to remove the seed coat. This action is achieved by stone-coated rollers [65].

7.3.5.2.8 Polishing

Polishing is done to provide a luster and improve consumer appeal. A screw conveyor is usually used for this operation. Depending on consumer need, different polishing materials, such as water, oil, or soapstone powder are applied to the split surface [56]. Products can then be packaged for consumption.

7.3.5.3 Pulse Flour Milling

Cleaned seed is ground into flour using a hammer, pin, and/or roller mill. Flours can be made from whole seed, dehulled whole seed, or dehulled split seed. Consumers usually prefer dehulled pulse flour, as the bitter flavor, typical of pulses, is minimized [59]. The flour is then packaged and sold to either the retail or ingredient supply market.

7.3.5.4 Fractionation

A relatively new opportunity in pulse processing is specialized wet and dry milling to fractionate pulses. This method generates products that can be sold as ingredients to food processors and other industrial users [59]. With respect to legume utilization, milling of legumes and fractionation of protein and starch have increased in recent years. Milling of whole seed or dehulled seed followed by fractionation of starch-rich and protein-rich fractions will improve the utilization of legumes [66]. The air classification technology is based on the utilization of a spiral air stream to differentiate fine fraction (protein) and coarse fraction (starch). In other words, the particles, which differ in density and mass, are separated in a current spiral airstream [67].

7.3.5.5 Milling Machinery

The loosened seed coats of the pretreated pulses are removed in the milling operation. For this purpose different machines are used, depending upon the type of pulse and scale of operation. The machines work on principles of (i) compression, (ii) shear, (iii) abrasion, and (iv) impact [61].

7.3.5.5.1 Hammer Mill

Hammer mills are composed of hammers mounted on a rotating shaft within a peripheral screen. Grain is subjected to impact forces from the hammers until it is milled fine enough to go through the screen openings. Thus screen opening size primarily controls the fineness of the grind. However,

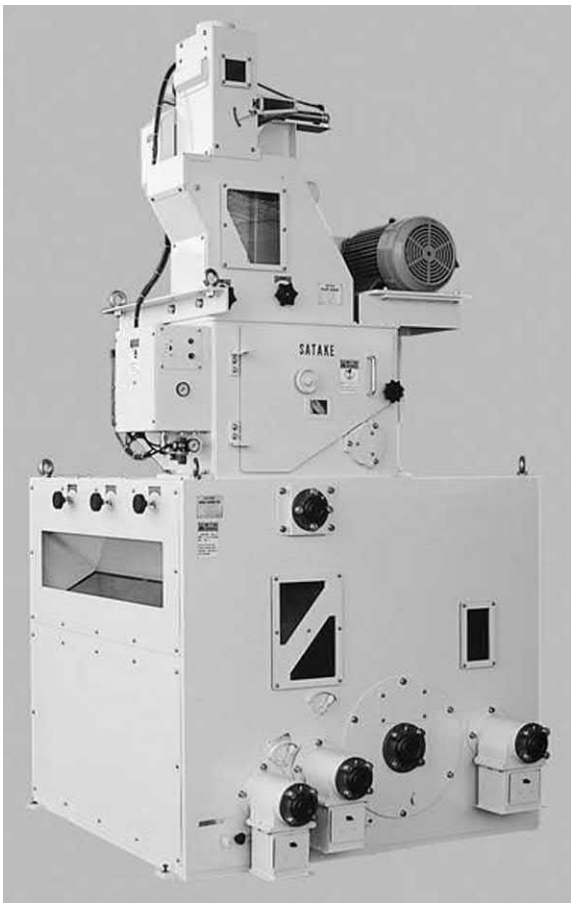

FIGURE 7.27 HR Paddy Husker. (From Stake Corporation [85].)

parameters such as hammer tip speed, rotor volume, type and number of hammers, and feed rate also influence the performance of the hammer mill [68].

7.3.5.5.2 Roller Mill

The roller mill consists of rotating corrugated or smooth paired cylindrical rolls oriented horizontally. Feed grain particles are reduced in size between the metal rolls. The grains are subjected to shear and compressive forces caused by the rolls when particles are pulled toward the nip [69].

7.3.5.5.3 Huller

Dehulling can also be achieved by using an impact huller, where grains are fed down the hollow axle of a rapidly rotating disc. The accelerated grains collide with the wall of the huller, which is typically lined with a polymer that reduces breakage compared with a metal surface.

7.3.5.5.4 Pin Mill

Impact disintegration is a kind of milling that can break up cellular materials selectively, without damaging the starch granules [70]. Traditionally, the pin mill, which is a kind of impact mill, is employed to disintegrate the starch–protein bond and produce fine flour. The pin mill is also categorized as a disc mill. It employs shearing and impact forces to produce fine flour by breaking up the dehulled seed [71]. The pins

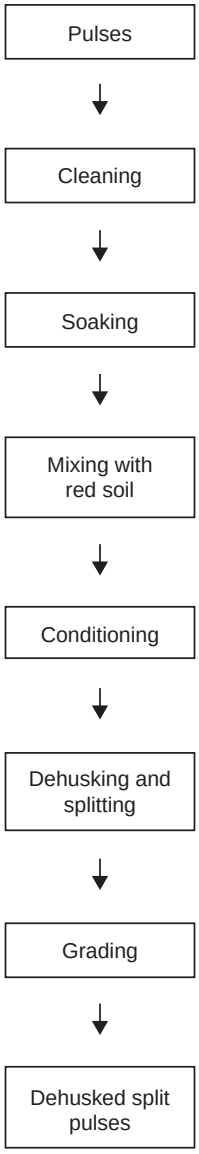

FIGURE 7.28 Flow diagram of pulse wet milling.

in a pin mill are fixed to two discs that rotate opposite of each other at different speeds (Figure 7.31). The radial speed leads to pins pressing the material at high speed through the rows of pins. The resultant impact force grinds the material. The fineness of the final product depends on the distribution and shape of the pins, the circumferential speed of the rotors, the feed rate, and physical properties of the material [72]. Pin mills may be used for dry or wet milling [71, 72]. Moisture content below 10% (w.b.) is optimal for milling of legumes [73].

Some pulses may be subjected to pin milling twice to obtain fine-grade flour [65]. The pin-milled flour is air classified in a spiral air stream and fractioned into light and heavy particles. The fine and light particles contain protein, whereas the coarse and heavier particles mostly contain starch granules [64]. The starch granules remain intact during pin milling and the process must avoid damage to the starch granules. Although dry processing (air classification) does not result in a pure protein fraction as wet processing, it is an effluent-free

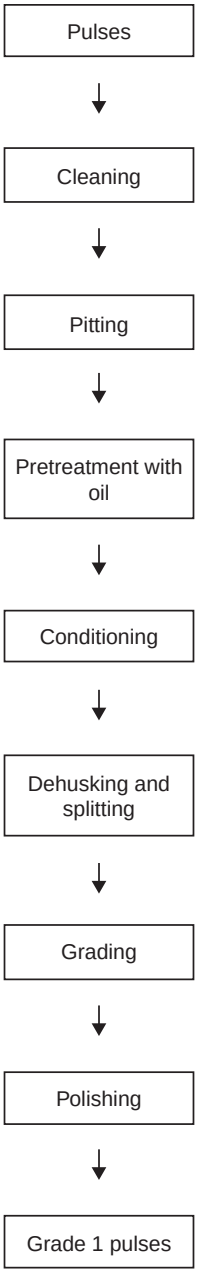

FIGURE 7.29 Flow diagram of pulse dry milling.

process and separated fractions are suitable as food, food ingredients, and other uses [74]. Since some protein bodies still adhere to the starch granules at the end of pin milling, it is necessary to reprocess the coarse fraction by pin milling and air classification for increased protein yield [65].

7.3.5.6 Pulse Milling

Most pulses are consumed in the dehusked, split form. Recovery of dehusked splits from pulses depends on the proportion of the husk to cotyledon and the way it is attached to the cotyledons. Pulse milling, as it is practiced today, is quite tedious, involving elaborate premilling treatments for loosening of the husk and also a long processing time. Traditional

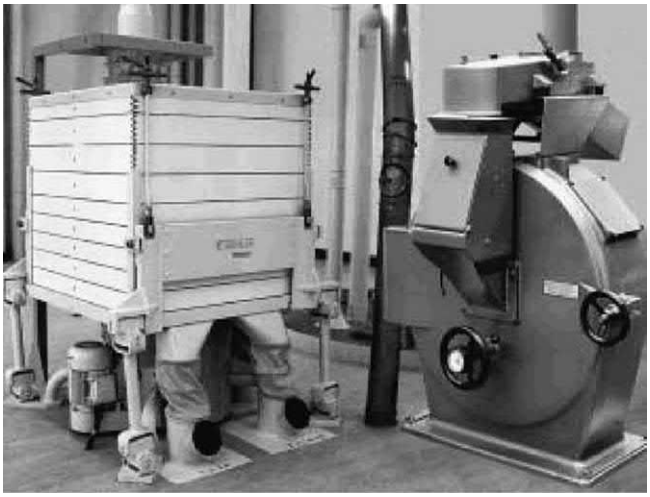

FIGURE 7.30 Equipment for dehulling and splitting pulses. (From Goodman [55].)

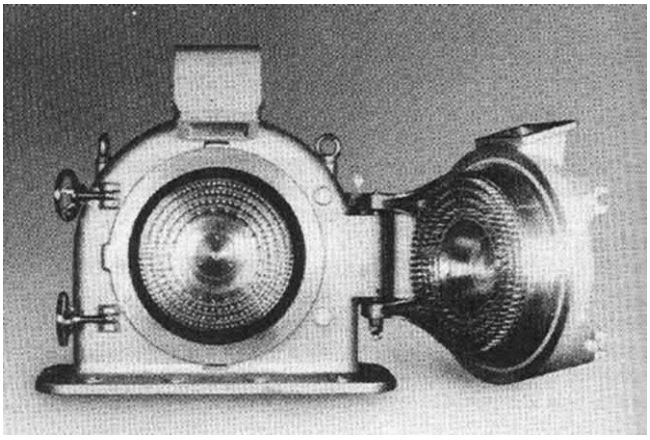

FIGURE 7.31 Pinned disc mill. (From Emami et al. [70]; Fellows [71].

pulse milling methods require more abrasive force due to improper preconditioning. Conservation of pulses can be achieved mainly through the development of superior milling procedures and equipment.

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8 Postharvest Handling and Preservation of Fresh Fish and Seafood

Umezuruike Linus Opara, Saud Musallam Al-Jufaili, and Mohammad Shafiur Rahman

CONTENTS

8.1 INTRODUCTION

The fisheries sector plays an important role in food security, food industries development, and poverty alleviation in many parts of the world. The consumption of fish and fishery products has been strongly influenced by improvements in postharvest handling, packaging, storage, transportation, and marketing. These have led to significant improvements in post-production efficiency, lower costs, wider product choice, and safer and improved products. Advances in marine and fisheries science and engineering as well as food product development have resulted in technological innovations for fish capture, postharvest handling, processing, marketing, and distribution. The extent and range of these changes have varied among regions; however, there is increasing recognition of the importance of fish and other seafood in the economies of many regions. The supply of food proteins to many parts of the developing world has raised awareness of the role of fish and other marine foods in enhancing food security and alleviating malnutrition and poverty. In general, there has been a growth in fish and fishery products marketed in fresh

form and in the production of ready-to-cook or ready-to-eat products, particularly in wealthy economies and due partly to increasing understanding of fish functionality in health. The development and application of efficient and cost-effective postharvest technologies for the handling and preservation of fresh fish and other seafood are therefore important to ensure product safety and maintenance of quality throughout the supply chain from sea to plate.

The aim of this chapter is to outline and discuss the techniques and procedures for postharvest handling and preservation of fresh fish and other seafood products. A wide range of marine organisms (fish, crustaceans, and mollusks) are consumed directly by humans as food or utilized as industrial raw materials such as animal feed. Each fish species may respond slightly differently to particular handling procedures and preservation techniques; however, this chapter focuses on the major technologies, which are applicable for the handling and preservation of a wide range of fresh fish and seafood. Where appropriate, specific technological innovations for improved handling, quality maintenance, and preservation are mentioned.

8.1.1 Fish and Fish Products

Processed fish for human consumption (frozen, cured, and canned) remained relatively stable at around 39 million tons. Freezing represents the main method of processing fish for food use, accounting for 53% of total processed fish for human consumption in 2002, followed by canning (27%) and curing (20%). In developed countries, the proportion of fish that is frozen has been constantly increasing, and it accounted for 42% of production in 2002. By comparison, the share of frozen products was 13% of total production in developing countries, where fish is largely marketed in fresh/chilled form. The high demand for fish as fresh produce (Figure 8.1) is partly explained by increasing consumer interest and understanding of fish and fish products as functional food. The high demand for fresh fish also underlines the increasing need to develop and apply appropriate innovative postharvest handling systems for the maintenance of fish freshness and product safety.

In 2002, the average apparent per capita consumption of fish, crustaceans, and mollusks worldwide was estimated to be about 16.2 kg, 21% higher than in 1992 (13.1 kg). This growth is largely attributable to China, whose estimated share of world fish production increased from 16% in 1992 to 33% in 2002. If China is excluded, the per capita fish supply would be 13.2 kg, almost the same as in 1992. Following a peak of 14.6 kg in 1987, world per capita fish supply, excluding China, showed a declining trend from the late 1980s to the early 1990s but has stabilized since then (Figure 8.2). The declining trend was mainly caused by population growth outpacing that of food fish supply during the 1987–2002 period (1.3% per annum compared with 0.6%, respectively). For China, the corresponding annual increase since 1987 was 1.1% for population growth and 8.9% for food fish supply. In 2002, per capita fish supply in China was about 27.7 kg.

8.1.2 Problem of Postharvest Losses in Fish and Seafood

Harvested fish and seafood materials undergo a series of handling operations from catch sites until the product is delivered to the end-user. Fish are highly perishable starting from the point of harvest. Several factors predispose fresh fish to rapid quality degradation once they are harvested, and these include alteration of the surrounding environment due to removal from the marine or aquatic environment, high moisture content of fish, activities of microorganisms inside the gut and intestine, and physical damage resulting from use of improper harvesting tools and procedures and rough handling practices. Apart from being a cosmetic defect, which results in downgrading of fresh fish quality, the presence of physical damage due to rough handling also predisposes fresh fish to accelerated water loss as well as opportunistic microbial infection during subsequent handling operations.

Postharvest losses in fish may occur quantitatively and/or qualitatively. Quantity losses (wastage) occur when fish is removed from the food chain, and this may occur due to spillage or discard when the product is considered completely unacceptable for utilization as food and as an industrial raw

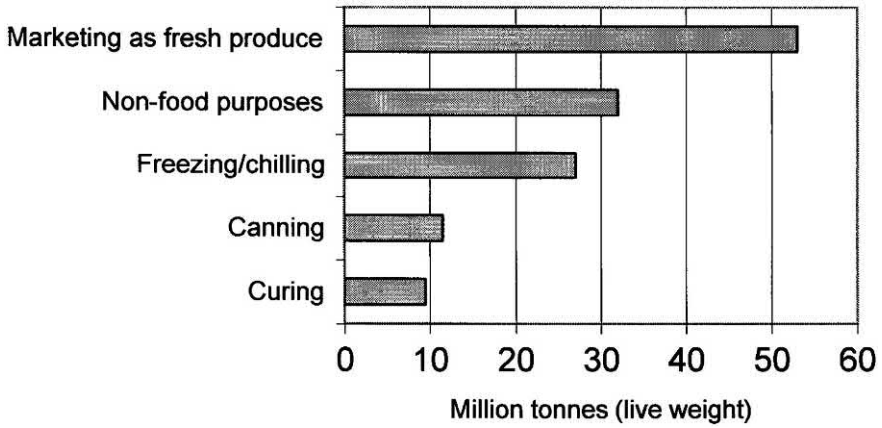

FIGURE 8.1 Utilization of world fisheries production (breakdown by quantity), 2002. (Adapted from Delgado et al. [33].)

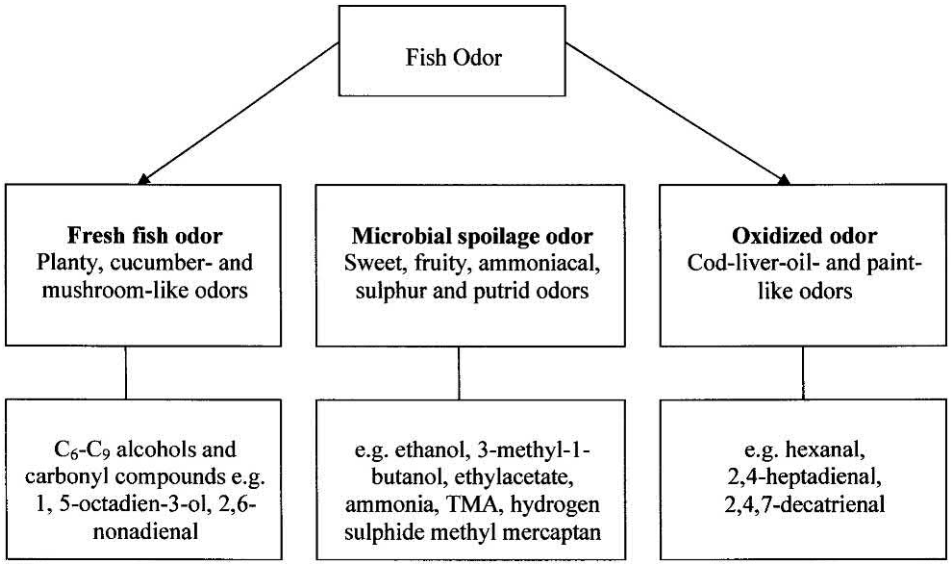

FIGURE 8.2 Categorization of fish odors and the volatile compounds that contribute to the characteristic odor of fresh, spoiled, and oxidized fish. TMA=trimethylamine. (From Olafsdottir et al. [41].)

material. Quantity loss may also be referred to as physical loss. Quality loss in fresh fish occurs when the product value to the end-user is compromised and downgraded due to a reduction in the attributes that are important to the end-user. Quality loss often results in a lower unit market price of fish products and a concomitant reduction in profits to the producer. Given the high volume of globally traded fresh fish produce, quality loss is a major contributor to total economic loss in the fresh fish and seafood industry.

The availability of reliable data on the magnitude and sources of postharvest losses is important in efforts to reduce the incidence of physical and quality losses in the fish and seafood industry. Exact data on losses of fish and other seafood materials are not readily available due to a myriad of factors including variations in species, geographical location, and season, as well as differences in harvesting, postharvest handling, and marketing systems. Nonetheless, several researchers have reported the incidence of fish losses based on surveys at various steps in the handling and marketing chain [1–4]. Others have developed empirical models of postharvest fish losses based on a consideration of the physical and monetary losses associated with various steps in fish handling from landing sites to export [5].

Global levels of fish losses are often estimated to be 20–40%. Published annual estimates of global fish losses include 3 million tons [2], 4.2 million tons [6], and 10–12 million tons [7]; however, most authors do not provide detailed information on the method of data acquisition and the specific type of losses reported. Nevertheless, these figures are expected to be much higher in many producing regions in developing countries where fish harvesting and postharvest handling technologies are not adequate to meet the needs of modern integrated long supply chains and marketing systems. In the Lake Victoria fishing community in Kenya with an annual catch of about 180,143 tons in 2000 and worth over 9 billion Kenyan shillings, it has been estimated that postharvest losses ranged from 20 to 25% [4]. These losses could double to 50% in some seasons or specific locations when large catches are made [8]. Further studies on three major fish species at seven beaches in that area reported quality losses of 13.9% for Clarias fishery, 6.4% for Nile perch, and 6.2% for Protoperus [4]. The authors identified the lack of basic cooling and preservation facilities at landing sites as major causes of quality loss.

Application of the generalized fish postharvest loss model by Cheke and Ward [5] to the Nile Perch fisheries in the Tanzania area of Lake Victoria showed that the total value of losses (US$) per 100 kg ranged from 4.20 to 7.55, depending on the type of fishing gear and method of transportation. The value of physical loss alone ranged from US$3.14 per 100 kg for fish caught by beach seine nets and transported by air to US$4.62 per 100 kg for fish caught by gill nets and transported by rail. According to Cheke and Ward [5], these model predictions supported the common knowledge among fishers and marketers that fish from beach seine nets are usually much fresher and of better quality when landed than those caught by gill nets or long lines.

In the Sultanate of Oman, the high incidence of postharvest losses has been recognized as an economic problem mitigating the economic development of the fisheries sector. The annual loss due to downgrading of fish was estimated at nearly US$62 million [9]. On fish type basis, the loss in quantity ranged between 40 and 70% for demersal fishes, 5% for the pelagic, and 10% for the entire traditional fisheries sector. Recent studies at major fish landing and marketing sites in the Muscat Governorate in the Sultanate of Oman [3, 10] showed that fresh fish losses were a common problem. Fishers and marketers reported a high incidence of losses, resulting in loss of potential revenue ranging from 12.5 to 20%. The authors also found that for the large pelagic, downgrading due to loss

in freshness (value) could reach between 10 and 25% after the first day of landing and 19.0 and 43.8% after the second day at the market.

8.2 MECHANISMS AND MANIFESTATIONS OF SPOILAGE IN FISH AND SEAFOOD

Fresh fish and other seafood undergo many chemical and biological changes immediately after capture, which can ultimately result in spoilage. Several factors contribute to such spoilage in fish, including interactions between the products and handling equipment, interactions between the product and the surrounding environment and atmosphere, and the inherent self-destructive biochemical changes that take place inside the fish once it is harvested. The occurrence of spoilage in fish is manifested and perceived by the end-user through changes in several sensory perceptions, including odor, color, shape, texture, and composition. In this section, we describe the biochemical mechanisms of fish and seafood spoilage, the factors contributing to the incidence of spoilage, and conclude with a synthesis of the various manifestations of spoilage in fresh fish and seafood products.

8.2.1 Biochemical Aspects of Fresh Fish and Seafood Spoilage

Prior to harvest, fish are protected by a skin that secretes antimicrobial compounds, such as lysozyme, and by antibodies in the blood. This self-protecting and self-regulating property is indicative of the biochemical composition of fish. For instance, lean fish contain 20% protein, less than 5% lipid, with little carbohydrate, whereas fatty fish contain 10–30% lipid. The pH of fish flesh is neutral, and the flesh is highly buffered due to the presence of phosphates and creatine in the muscle and has a low oxidation-reduction potential [11].

The harvesting of marine, fresh-water, or aquaculture fish and other seafood is an essential step in the delivery of desired products to the consumer. However, harvesting results in the death of fish with the following consequences [11]: (i) cessation of energy supply for normal body function; (ii) cell membranes are no longer energized, and molecules and ions can freely diffuse; (iii) antimicrobials are no longer produced or distributed; and (iv) microflora penetrates the skin from outside surface and flesh from the intestines and gills.

During this period, the contractile mechanism can still operate, permitting the muscle to contract and relax [12]. In order to remain relaxed, the muscle consumes adenosine triphosphate (ATP); thus, when ATP is no longer sufficient the muscle will contract, resulting in a phenomenon called rigor mortis. In broad terms, rigor mortis is the change that occurs in muscle following death, and its development is influenced by several factors including (i) the prevailing environmental conditions, notably temperature (increasing temperature accelerates it), (ii) the health status of the fish prior to harvest, and (iii) any reduction in muscle glycogen during life. The physical and physiological symptoms of rigor mortis include (i) stiffening, hardening, and shortening of the muscle, (ii) loss of transparency, and (iii) loss of elasticity. In iced fish, this condition usually lasts for one day or more, followed by the resolution of rigor.

All muscle contracts in rigor mortis, either because ATP is exhausted or because the pH has fallen sufficiently below the critical level. Suzuki [13] studied the linewidths of NMR spectra of water in flat fish in pre-rigor, rigor, and post-rigor stages. The results showed that line width in the rigor stage was broader than in the pre-rigor and post-rigor stages, but in the post-rigor stage, it became narrower than in the pre-rigor stage. The same results were also observed in sea bass, but only in the case of starved carp where rigor mortis was obscure and the change in the width of lines was not clear [11]. The state of water in muscles as a function of time after death by spin–lattice relaxation showed that during rigor there was a net transfer of water from the free phase of the region, giving rise to a more rapidly relaxing signal [14].

Marine fish contain trimethylamine oxide (TMAO) as an osmoregulator. Some bacteria or endogenous enzymes can reduce TMAO to trimethylamine (TMA, which has the odor of stale fish) and formaldehyde. The bacteria can obtain energy from this reaction and use reduced TMAO as an electron acceptor in the absence of oxygen. Fresh-water fish containing TMAO usually have a longer shelf life, even if the level of TMAO is low. Thus, the level of TMAO-reducing bacteria is important in order to preserve marine fish. There is little fermentation activity in the microflora due to the low carbohydrate content of most fish [11]. The initial quality of seafood is related to the species, the growing area conditions, the fishing or harvesting techniques, the seasonal biological changes in muscle and other organs, and the postharvest storage and processing conditions [15]. The ultimate quality is linked to the biochemical changes in the major constituents and microbial load and type in the fish or seafood and water from which they are harvested. Bacteria cause organoleptic changes. The active bacteria are psychrotrophic or cold-loving and well-adapted to growth under chill conditions [11].

Stress may occur even at the stunning and killing phases of fishing. A very rapid drop in muscle pH due to stress at killing can affect color parameters (high lightness, hue, and chroma values). This makes fish flesh lighter and more opaque than that of unstressed fish [16]. In addition, increased depletion of muscle energy, anaerobic glycolysis, and lactate accumulation occur due to high muscle activity during slaughter, and these cause a reduction in pH [17]. Electrical stunning (E) and asphyxia in the air (A) are two common stunning/killing methods for fish [18]. The effects of carbon monoxide asphyxia (CO) were compared to E and A on the evolution of post-rigor mortis changes in frozen rainbow trout fillets related to chemical, textural, and sensorial properties during storage time. Fish fillets from the E group showed a higher pH than the A group, with the CO group being intermediate. The CO treatment ensured that the fillets showed the lowest yellowness index while lipid oxidation and texture profile analyses were unaffected, and sensory analysis revealed that

the CO fillets had the lowest odor intensity with the highest juiciness scores [18].

8.2.2 Characterization and Quantification of Fish Spoilage

The most distinct stages of fish spoilage are shown in Table 8.1, based on results obtained from codfish stored in ice over an extended period of time [19]. In summary, a combination of visible signs of degradation and odor production may be used to characterize different phases of spoilage after harvest.

Cooked flavor without added condiments is the most precise objective method of assessing fish spoilage and quality changes. For practical purposes, however, this destructive approach is time-consuming and not suitable for rapid assessment during postharvest handling and marketing operations. For non-destructive measurement, changes in gill color, gill odor, eye color, skin-color, skin-rubberiness, and odor production may be used alone or in combination, to assess the quality status of fresh fish. Among the various non-destructive testing approaches, gill odor has been considered the most reliable and reproducible characteristic [11].

Several biochemical changes have been successfully used to quantify the level of spoilage in fresh fish and seafood. The concentration of TMA is used as an index of spoilage either alone or as a component of the total volatiles containing ammonia and other amines. An alternative chemical index of fish spoilage is hypoxanthine, which is derived from the breakdown of adenosine triphosphate. After the death of a fish, ATP in the meat decomposes to uric acid by a series of catabolic enzymes [20]. Hypoxanthine is an intermediate of these reactions and accumulates as storage time increases. Another more complex measurement is the potassium value, which is based on ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), and hypoxanthine [11]. Changes in the dielectric properties of whole fish and skin-on portions occur during spoilage, and these changes can be measured in a non-destructive manner [11].

Drip loss is a quality problem in fish and seafood products. Once the water-holding capacity falls below the water content of the muscle, excess water will be lost, either immediately or later during processing or cooking [12]. Reducing drip loss during postharvest handling and marketing is therefore important in the value-chain management of fish and other fresh foods. The amount of drip loss from muscle depends largely upon changes in the water-holding capacity of the muscle protein after death. The magnitude of drip loss is influenced by a combination of the time and temperature of the immediate postmortem period [12]; therefore, adequate cold-chain management is critical in reducing the incidence of drip loss.

In summary, postharvest fish muscle quality can be maintained and spoilage reduced by a delay in onset of rigor mortis (associated with low stress) or the use of rapid killing methods [20]. In addition, Jerret et al. [20] have noted that a combination of behavioral conditioning, conservative handling practices, and chemical anesthesia can be used to minimize the extent of premortem exercise and thereby provide rested fish. The authors also recommended the importance of reducing preharvest exercise in the production of high-quality fish muscle.

TABLE 8.1
Characteristics of Phase Changes during Spoilage of Cod Fish Stored in Ice

Phase	Storage (Days)	Characteristic Changes
I	0–6	No marked sign of spoilage
II	7–10	No odor production
III	11–14	Production of some odor, slightly sweet to fruity odors
IV	>14	Production of hydrogen sulfite and other sulfide compounds, fecal, and strong ammonia odors

Source: Shewan [19].

8.2.3 Abiotic, Biotic, and Physiological Causes of Fish and Seafood Spoilage

Spoilage of fish and seafood is caused by a myriad of factors, which are related to the harvesting and handling practices and equipment, postharvest handling environmental conditions, the presence of decay-causing microbiological agents, and internal physiological changes or biochemical reactions that are associated with the normal process of aging and death. Like other fresh produce and irrespective of the primary causative agent, the rate of spoilage and quality degradation of fresh fish and seafood products is highly accelerated at elevated temperatures. Conversely, the deleterious effects of spoilage-causing agents can be significantly reduced through a synergistic effect of adequate temperature management in combination with other specific measures.

8.2.3.1 Mechanical Handling Damage

Wholesomeness and freedom from physical damage are important quality attributes in the fish trade, especially for fresh fish destined for the consumer. During harvesting and postharvest handling operations, fish may be subjected to excessive forces, which result in physical injury and blemish. Such damage may occur through several mechanisms including impact, compression, friction/abrasion, and cuts. Impact damage occurs when the fish falls from excessive height to generate sufficient absorbed impact energy that causes damage to the skin alone or in combination with underlying flesh. Compression damage occurs due to excessive stationary forces acting on the produce (such as cartons of produce stacked too high). Mechanical handling of fish affects the degradation of nucleotides in fish. Moreover, handling and mechanical damage to muscle tissue may increase the degradation of inosine monophosphate and inosine by making their substrates more accessible due to enzyme compartmentalization [21]. Furthermore, the presence of physical injuries such as bruises, cuts, and abrasions, provides favorable sites

for opportunistic infection and contamination of produce by decay-causing microorganisms.

8.2.3.2 Environmental Factors

Undesirable environmental conditions around fresh produce, such as the degree of hotness or coldness (temperature), water vapor content in the air (relative humidity), as well as direct exposure to sunlight and airflow, contribute to the onset and rate of quality deterioration and spoilage. By far, temperature is the most influential factor affecting spoilage of fresh produce such as fish and seafood. In addition to its effects in accelerating physiological and biochemical processes associated with tissue degradation and eventual spoilage, temperature also accelerates the action of microbial agents (such as bacteria), which cause spoilage and food safety hazards. The freshness of high-water-content foods such as fish and seafood is maintained better under high relative humidity conditions; thus, storing such produce at low relative humidity contributes to rapid spoilage and development of undesirable attributes. Airflow around fresh fish and seafood must be closely monitored to avoid excessive loss of surface moisture and undesirable changes in skin and flesh quality.

8.2.3.3 Biotic (Bacterial) Factors

The deterioration of fresh fish is primarily due to bacteria action. It is recognized that only some of the bacteria present are responsible for producing the off-odors, off-flavors, appearance, and textural changes that constitute spoiled fish [11]. The composition of the microflora is dependent on different factors, such as environment and season. Spoilage bacteria affect the degradation of nucleotides in fish. In cod fillets, the presence of spoilage bacteria increased the rate of degradation of inosine to hypoxanthine. This indicates that bacterial enzymes play an active role in contributing to the degradative process. The synthesis of proteases depends on the nutrient source, and secretion of proteases increases with the decrease in available nutrients, reaching a maximum concentration by the late log or early stationary phase of the bacterial growth. The level of protease secretion is low during the initial stage of bacterial spoilage, i.e., 106 CFU/g of flesh. Above 106 CFU/g there is high protease secretion [21].

Bacterial spoilage is evident in fish even at 0 to –4°C, but spoilage can be prevented below –10°C [19]. For cod stored in ice, there is a 2- to 3-day lag period with a logarithmic increase by day 10 in the bacterial flora, generally with counts up to 108/cm^2 skin or 108/g muscle. Pseudomonas species dominate up to 90% by day 12. They produce the spoilage odors, such as ammonia, and volatile sulfur compounds, such as mercaptans and hydrogen sulfide [22].

Consumption of contaminated fish and seafood is a potential source of health and safety hazards to humans. The main hazards of fish and seafood are pathogenic bacteria (aquatic environment, from humans or animals, biogenic amine producers, spoilage bacteria), parasites, biotoxins, and viruses [23]. All aquatic environments can harbor spores of *Clostridium botulinum*, which can contaminate fish both in marine and freshwater environments. *Vibrio parahaemolyticus* is the lead cause of food poisoning in Japan, where much fish is eaten raw. It does not grow below 10°C and dies out at chill temperatures, and is heat-labile. As it is halophilic, it survives salting and smoking. Control is therefore achieved by proper chilled storage. There has been a global increase in the presence of algal toxins in shellfish. More toxins are being identified. One of the major concerns in recent years for the fish processing industry has been *Listeria monocytogenes*. This organism can grow at chill temperatures, and it is not inhibited by the levels of salt in smoked products [11].

The bacterial flora of water reflects the flora of fish harvested from the area. The flora of cold-water fish is predominantly gram-negative, while flora in tropical fish are predominantly mesophilic gram-positive microorganisms [21]. The dominant microflora of cold-water fish species are *Pseudomonas*, *Alteromonas*, *Moraxella*, *Acinetobacter*, *Vibrio*, *Flavobacterium*, and *Cytophaga* [24]. Gram-positive organisms found on warm fish species are predominantly *Micrococcus* and *Bacillus* [21]. Psychrotrophic bacteria are found in almost all types of refrigerated and frozen foods. The outgrowth of pseudomonads in spoiling fish is due to their efficient use of free amino acids (especially methionine and cysteine) and peptides in the non-protein fraction during the early stage of spoilage, and their secretion of potent proteases, thus promoting proteolysis after low molecular weight components have been exhausted. The primary use of all nitrogenous compounds is through oxidative deamination, which results in the accumulation of ammonia, volatile fatty acids, and sulfur-containing compounds [24]. Volatile sulfur-containing compounds are believed to produce spoilage odors. These compounds have extremely low thresholds, and their origin is usually traced to non-protein nitrogenous compounds in the skin and flesh.

Changes in *K* value index (ratio of inosine and hypoxanthine to the quantity of ATP), ADP (adenosine diphosphate), AMP (adenosine monophosphate), and IMP (inosine monophosphate) are an objective measure of fish muscle quality during postharvest handling and storage. The *K* value is calculated as [25]:

$$K = \frac{HXR + HX}{ATP + AMP + IMP + HXR + HX} \times 100$$

where *HXR* is hypoxanthine riboside, *HX* is hypoxanthine, and *IMP* is inosine monophosphate. The storage temperature effects on histamine production, for example, fresh yellowfin tuna maintained an acceptable shelf life based on the permitted limit of *K* value for 12, 5, and 1 days at 0, 8, and 20°C, respectively [26]. The fish was rejected by panelists earlier than their *K* value indicated. The fish stored at 8 and 20°C became unsafe for human consumption, reaching unacceptable histamine levels after 4 and 1 days, respectively.

Some volatile compounds that have been isolated from spoiling fish muscle are ethyl mercaptan, methyl mercaptan, dimethyl sulfide, hydrogen sulfide, acetaldehyde, propionaldehyde, diacetyl ethanol, methanol, acetone, acetoin, butanal, methyl butanal, and ethanal [24]. Selected volatile compounds

TABLE 8.2
Selected Volatile Compounds and Their Probable Source in Spoiling Fish

Compound	Probable Source
Hydrogen sulfide	Cysteine
Dimethyl sulfide	Methionine
Methyl mercaptan	Methionine
Acidic, propionic, butyric, and hexanoic acid esters	Glycine, leucine, serine
Trimethylamine	Trimethylamine oxide
Dimethylamine	Triethylamine oxide
Ammonia	Urea, various amino acids

Source: Martin et al. [24].

in spoilage fish and their probable sources are compiled in Table 8.2.

8.2.3.4 Physiological (Internal) Factors: Lipid Oxidation and Hydrolysis

Lipid oxidation is a major cause of quality deterioration in fish and seafood due to lipid content and the extent of poly-unsaturation [24, 27]. Catalysts for lipid oxidation are molecular and singlet oxygen, metals such as iron and copper, and enzymes such as lipoxygenase [15]. The postmortem changes in fish muscle related to lipid oxidation are (i) decrease in ATP, (ii) increase in ATP breakdown products, e.g., hypoxanthine, (iii) changes in xanthine dehydrogenase to xanthine oxidase, (iv) loss of reducing compounds, e.g., ascorbate, glutathione, (v) increase in content of low molecular weight transition metals, (vi) conversion of heme (Fe II) pigments to oxidized form (Fe III), (v) loss of structural integrity membranes, (vi) loss of antioxidants in membranes, e.g., tocopherols, and (vii) inability of muscle cells to maintain calcium gradients [27].

These changes make the tissue more susceptible to oxidation, especially through changes in membranes. In addition, size reduction, such as fillets and minces, can enhance lipid oxidation by exposing more lipid to oxygen. The destruction of antioxidants such as tocopherols by heat, enzymes, and salts also plays an important role in lipid oxidation [15]. The glutathione peroxidase activity is located in various fish muscles and presumably protects muscle from oxidative deterioration of lipid during storage and processing [28]. Nakano et al. [29] reported that glutathione peroxidase increased significantly during storage, which suggested that the increase in enzyme activity could protect fish muscles from oxidative deterioration during storage and processing. Watanabe et al. [28] reported that total glutathione peroxidase activity in Japanese jack mackerel and skipjack tuna fish muscles decreased gradually during storage at 4°C.

Technological approaches to minimizing lipid oxidation that are applicable to fish and seafood include (i) reducing oxygen access to the product through controlled atmosphere storage and modified atmosphere packaging (e.g., vacuum packaging, edible coating), (ii) maintaining natural antioxidants or adding antioxidants, (iii) minimizing increases of pro-oxidants (e.g., iron), (iv) maintaining low temperatures through cool- and cold-chain management, (v) minimizing salt constituents, and (vi) removing unstable lipids (e.g., subcutaneous fats) and dark muscle, which contains more fat [15].

8.2.4 Physicochemical Manifestations of Spoilage in Fish and Seafood

When fresh fish and seafood products undergo improper handling and spoilage, certain changes occur in both the appearance and chemical composition, which can be readily quantified. Proper understanding of these changes is important for the early detection and isolation of affected produce. These manifestations of spoilage are largely sensory in nature.

8.2.4.1 Color Changes

Quality loss of fish muscle after harvest may take the form of color (appearance) changes. Fresh fish has a translucent appearance due to even scattering of incident light. With an increase in spoilage, there is a gradual disintegration of myofibrils, resulting in their wider and more random intracellular distribution. The fish surface then appears opaque because the incident light is unevenly scattered [21, 30].

Changes in fish flesh color occur during low-temperature and freezing storage. The flesh becomes yellow due to oxidation of carotenoid pigments and lipids in tissues. Other factors that may result in yellowing are lipid oxidation and reactions with carbonyl-amines. The red color changes in whole orange roughy during ice storage are shown in Table 8.3 [21].

Factors affecting pigment loss are as follows: (i) myeloperoxidase from fish leukocytes causes rapid discoloration of β-carotene in the presence of hydrogen peroxide and iodide or bromide ions due to the breakage of double bonds, (ii) free radicals addition, free radical abstraction, or singlet oxygen addition to the double bonds of β-carotene, (iii) temperature

TABLE 8.3
Color Changes and Appearance of Gills in Whole Orange Roughy Stored in Ice

Storage (Days)	Color Changes	Appearance of Gills
0	Red/orange	Dark red
4	Red/orange	Dark red
6	Orange on fins, head, and tail	Dark red
9	Slightly blotched, body faded	Dark red, slightly milky slime
11	Blue steel-gray with tinges of orange	Brown/red or bleached, sticky, creamy, slime
13	Bleached pale gray or blue tail with head pale orange	Brown/red or bleached with brown slime on gills
16	Washed-out gray/blue with pale head	Brown or bleached with brown slime

Source: Scott et al. [103].

TABLE 8.4
Appearance of Surface Slime on Whole Orange Roughy Stored in Ice

Storage (Days)	Color and Consistency of Surface Slime
0	No slime
4	Clear slime
6	Clear or slightly cloudy slime
9	Clear or slightly cloudy slime
11	Clear or slightly cloudy slime
13	Brown slime on body
16	Thick yellow slime

Source: Scott et al. [103].

and concentration of storage oxygen, and (iv) progressive increase in pH from 7 to 8 during storage: potassium β–oxyacrolein, the enolic salt of malondialdehyde, formed self-condensation reactions and polymerised at pH 7–8 to form fluorescing compounds [21]. The appearance of surface slime on whole orange roughy as a function of storage duration in ice is shown in Table 8.4.

8.2.4.2 Texture Changes

Fresh fish has characteristic firmness, which can be rapidly assessed subjectively by hand feel, and objectively by instrumental test and sensory panel test. During postharvest handling and storage, the texture of fresh fish changes from "firm" and "moist" to "mushy" and "runny." These textural changes occur due to tissue softening as a result of myofibrillar disintegration and the weakening of connective tissue. During storage, the spoiling intracellular and extracellular proteases degrade myofibrillar proteins [21]. The loss of firmness and resilience and the development of unpleasant odors and flavors are some of the quality changes that occur in fish and seafood after harvest. The changes in texture and odor of orange roughy (i.e., whole) stored in ice for 16 days are shown in Table 8.5. The sensory changes of white fish are presented in Table 8.6. Manifestations of quality degradation and spoilage of stored fish muscle may take the form of excessively soft tissue, loss of liquid-holding capacity, and development of a dry or rough texture upon cooking [31].

Postmortem tenderization of fish muscle is one of the major problems related to fish freshness and its quality [32]. The causes of postmortem tenderization postulated so far are as follows [33]: (i) a weakening of rigor between myosin and actin as seen by the decrease of Mg^{+}-ATPase activity, (ii) breaking down of Z-disc structure of myofibrils, and (iii) degradation of titin [32].

Ando et al. [34] also suggested that postmortem tenderization of fish muscle is closely related to the gradual disintegration of the extracellular matrix structure after death. The weakening of the Z-line depends on a proteolytic mechanism, which removes α-actinin from this structure by action of a calcium-dependent neutral proteinase: calpain [32]. In addition, other myofibrillar proteins such as titin and nebulin

TABLE 8.5
Changes in Odor and Texture of Whole Orange Roughy Stored in Ice

Storage (Days)	Odor Changes in Gills	Texture Changes in Flesh
0	Slightly seaweedy	Firm and resilient
4	Slightly seaweedy	Firm and resilient
6	Mild and sweet	Firm and resilient
9	Fish meal, sweet, salty, briny	Firm and resilient
11	Sweet, salty, mussel, soapy, oily	Retains finger indentation, no gaping, slightly soft
13	Metallic, stale. Seaweedy and slightly rotting odors	Retains finger indentation, soft
16	Strong rotting and putrid	Retains finger indentation, no gaping, soft

Source: Scott et al. [103].

TABLE 8.6
Sensory Changes in White Fish

Quality	Score	Raw		Cooked	
		Gill Odor	General Appearance	Odor or Flavor	Texture
Fresh	10	Seaweed, sharp metallic	Convex eyes, shiny red blood, flesh translucent	Slight sweet/ meaty	Dry
Spoiling	7	Bland, loss of tanginess	Eyes flat, dull skin, no translucence	Bland/ neutral	Firm/ succulent
Stale	5	Milky, mousy, yeasty	Sunken eyes, browning of gills, opaque mucus, waxy flesh	Some amines	Softening
Putrid	3	Acetic acid, old boots odor, sour stink	Eyes sunken, yellow flesh, brown blood, yellow bacterial lime	Bitter, sulfites	Mushy

Source: Gibson [11].

are also implicated in postmortem weakening of fish [35] and meat muscle [36]. Papa et al. [32] studied α-actinin release and its degradation from myofibril Z-line in postmortem white dorsal muscle from bass and sea trout stored at 4°C and 10°C. Using α-actinin-specific antibodies, they showed that this protein is rapidly released within the first 24 hours for the two specific species, and reaches a plateau within 4 days. The release and proteolysis of α-actinin are time- and temperature-dependent processes that take place at early

stages of fish storage. The proteolysis of α-actinin seems to be dependent on fish species.

In fish intramuscular connective tissue, type I and V collagens are present. Sato et al. [37] demonstrated that type V collagen was solubilized specifically in softened rainbow trout muscle. Similarly, type V collagen became solubilized in softened sardine muscle after 1 day of chilled storage, whereas tiger puffer muscle did not show significant softening, changes in the structure of connective tissues, or biochemical properties of collagens [37]. This was due to the presence of more type I collagen in tiger puffer muscle than in sardine, carp, and mackerel [37]. Thus, degradation of type V collagen caused disintegration on the tin collagen fibrils in pericellular connective tissue, weakening pericellular connective tissue, and resulting in postharvest softening. Similarly, Tachibana et al. [38] reported that the degradation of Z-discs of ordinary muscle was faster in cultured red sea bream than in its wild counterpart.

This liquid-holding capacity of muscle is highly influenced by fibril swelling/contraction and the distribution of fluid between intra- and extracellular locations [39]. Moreover, changes in muscle structure are an important factor. Changes in muscle structure are strongly influenced by temperature, ionic strength, and chemical composition due to season, and maturation of muscle and pH. Ofstad et al. [31] concluded that the liquid-holding capacity of raw fish seemed to be dependent on two main factors: genetic differences in muscle protein, and the postmortem muscle pH and subsequent time-dependent muscle degradation. They found that salmon muscle possessed much better liquid-holding properties than the cod muscle, as did wild cod compared to fed cod regardless of the storage time. The myofibrils of the salmon muscle were denser and fat, and a granulated amorphous material filled the intra- and extracellular spaces. The denaturation characteristics of myosin, actinin, and a sarcoplasmic protein differed between salmon and cod, indicating the stability of the myosin–actomyosin complex. Postmortem degradation of the endomysial layer and the sarcolemma may further facilitate the release of liquid. Thus, the release was related to species-specific structural features and better stability of the muscle proteins. The severe liquid loss of fed cod was due to a low pH-induced denaturation and shrinkage of the myofibrils [31].

Holes and slits appear between the myotomes (muscle segments) because of breakage of the minute tubes of connective tissue from the myocommata (connective tissue sheets) and run between and around the muscle cells. This phenomenon is known as gaping in the musculature of fish [40]. A low pH leads to gaping and vice versa [40]. At a given temperature, the gaping increases with time, and subsequent freezing increases it further.

8.2.4.3 Odor Changes

As indicated earlier, the release of volatile compounds is one of the important indicators of freshness and spoilage of fish and seafood. Olafsdottir et al. [41] classified the volatile compounds contributing to fish odor into three groups based on origin, as shown in Figure 8.2. There is increasing interest in the importance of rapid measurement of volatile compounds in fresh fish and seafood as objective indicators of freshness. Several authors have reported the traditional methods that rely on classical chemical analysis for the study of total volatile bases and trimethylamine (TMA) in fish [21, 36, 42–45]. Future measurement innovations in this area appear to focus on the development of electronic noses [46, 47].

8.2.4.4 Protein Changes

Proteins in fish and seafood are subjected to significant changes during postharvest handling, storage (both iced and un-iced), and processing. The proteinases are responsible for changes, such as hydrolysis, which can result from animals or from spoilage microflora especially during later stages of spoilage [30]. The types of proteases and their inhibitors are shown in Table 8.7. The solubility of proteins during washing increased when fish were held for a longer time and/or at higher temperatures. This is because the degradation of myosin heavy chain and actin increased rapidly at longer storage times and/or elevated temperatures, resulting in a higher loss of total protein during washing. Lin and Park [48] studied the effect of postharvest storage temperatures and duration on proteolysis of Pacific whiting. Myosin heavy chain degraded rapidly during postharvest storage at low temperatures (0–5°C), and greater degradation occurred at elevated temperatures. Actin degradation was similar to that of myosin heavy chain but to a lesser degree. Degradation of both was highly correlated to protein solubility. Low temperatures reduced but did not completely inhibit proteolysis [48].

It is well-established that the myofibrillar proteins differ in stability depending on the habitat temperature of the species [49]. During iced and frozen storage, the thermal characteristics of myosin subunits deteriorate faster in cold-water than in warm-water fish [50, 51]. Venugopal [51] studied the sites of attack by proteases on protein and found that the points of cleavage for carboxypeptidase, aminopeptidase, endoprotease, and proteinase were carboxyl-terminal, amino-terminal, and internal peptide bonds, respectively.

TABLE 8.7
Types of Proteases and Their Inhibitors

Protease	Inhibitor
Serine protease	**Phenylmethyl sulfonyl fluoride**
	Diisopropyl fluorophosphate
Thiol protease	**Heavy metal iodoacetamide**
	N-ethyl maleimide
	Anipain
	Leupeptin
Metalloprotease	**EDTA**
	O-Phenanthroline
	8-Hydroxyquinoline
Acid protease	**Pepstain**
	Diazoacetyl norleucine methyl ester
	Epoxy (*p*-nitrophenoxy) propane

Source: **[51].**

Large amounts of glycogen in mammalian muscle can result in low final pH. The high the postmortem temperature, the quicker is the onset of rigor, the shorter its duration, and the more severe the contraction. However, if meat is chilled below about 13°C soon after death, severe contraction takes place almost immediately, resulting in permanent toughening of the meat. This is cold shortening [12].

8.3 POSTHARVEST TREATMENTS AND PRESERVATION OF FISH AND SEAFOOD

The maximum freshness of harvested fresh produce such as fish and seafood is immediately after harvest [52]. Beyond harvest, the adoption of improved handling systems, maintenance of the cold/cool chain, and application of appropriate physical and biochemical treatments are necessary to reduce the incidence of losses, preserve/maintain quality, and extend storage and shelf life. Warm- and cold-water fish do not always respond in the same way to postharvest handling practices and may often need different treatments to achieve the desired results [12]. Unlike meat, the global fish harvest destined for marketing is still largely hunted traditionally although the contribution of farmed fish (aquaculture) is increasing rapidly, particularly in Asia [33]. This approach gives limited scope for manipulating the immediate pre- and postmortem conditions. In the case of cold-water fish, higher temperatures accelerate the onset and resolution of rigor as in meat, but without resulting in cold-shortening. Another difference between the handling and preservation techniques of fish and animal meat is that fish never seem to attain such low pH values as are sometimes encountered in meat.

8.3.1 Improvement of Harvesting and Postharvest Handling Systems

Fish and seafood quality is affected by harvesting and postharvest handling techniques and equipment used. Improper harvesting and containerization may result in physical injuries such as bruising, cuts, and abrasion. It is also important that steps are taken to land the catch as soon as possible after harvest because the physiological processes that result in spoilage commence immediately. To reduce the incidence of physical damage, harvesting equipment and containers must be checked regularly to avoid the presence of sharp edges. Avoiding excessive loading of fish on top of each other or over-filled boxes of fish, particularly the large fish species such as tuna and kingfish, can reduce compression damage. During transportation from catch to landing sites, fish and seafood products need protection against direct sunlight and heat, particularly in tropical and sub-tropical environments where rapid increases in air temperature can occur in a short time. Throwing fish into containers or onto heaps should be avoided due to increased susceptibility to handling damage and subsequent spoilage.

Hanpongkittikun et al. [53] demonstrated the importance of good harvesting practices in seafood quality. The authors identified *Staphylococcus aureus*, *Salmonella*, and *Vibrio parahaemolyticus* from shrimp samples during ice storage. Using samples taken from controlled harvesting and the open market, the authors found that controlled harvested shrimp had a shelf life of 8 days, while samples from the open market had only 4 days of shelf life.

8.3.2 Pre-Storage Treatments

Pre-treatment of fish and seafood immediately after harvest and/or landing is necessary to improve sanitation and to remove the inedible portions, which would otherwise contribute to accelerated aging and spoilage. Pre-treatments such as washing and cleaning reduce contamination of physical debris and microbial organisms, which pose health and safety hazards to the consumer. Furthermore, gutting and bleeding are typical primary pre-processing treatments that are commonly carried out on fresh fish and some seafood.

Fish may be bled and gutted (i.e., their intestines removed) prior to subsequent handling storage. Depending on the end-use and market requirements, the fish head may also be removed. Gutting is essential for some species, otherwise the digestive enzymes and the bacteria in the gut would soon attack the flesh. In some species, this may not be necessary due to the anatomical location of the gut cavity relative to the edible parts [11]. It is customary to wash fish to remove blood and any remnants of guts. However, washing can remove some natural antimicrobial secretions and may not be advantageous to the storage life [11].

The limited efficacy of heat treatments directly applied to whole fish by hot water immersion or wash is probably due to slow heat penetration and/or changes in microflora interactions [54]. Vaz-Pires et al. [54] took 80 bacterial isolates from sea scad to study their heat stability and found *Shewanella colwellinia* to be more heat resistant. The main target site of damage from heat treatment tested in their experiments was the cell wall. Good hygiene practices are also needed to reduce the possibility of contamination of fish and seafood products. This is achievable on land, but it is difficult on-board fishing vessels [11].

8.3.3 Cold/Cool Chain Technology

Rapid removal of field heat after harvest and maintenance of the cold/cool chain is an effective strategy for fish and seafood preservation and quality maintenance. Chilling can be performed by refrigerated seawater (RS) (i.e., seawater is chilled by a refrigeration system); chilled seawater (CS) is the combination of ice with seawater, and slurry ice (SI) consists of crushed or generated ice of 250–500 μm in diameter. CS is more effective than RS because of the large heat capacity of ice [55–57]. The effectiveness of SI can be due to the following reasons: (i) the large heat capacity of ice is combined with micron-sized ice crystals maintaining a biphasic system for a longer time due to lower buoyancy [59–61]; (ii) the improved heat transfer due to longer contact time with ice as the ice is suspended; (iii) the higher contact surface area

of ice crystals with fish enhances heat transfer; (iv) it reduces fish surface damage due to lower abrasion; and (v) it enables more hygienic handling since slurry can be pumped easily [58]. Keys et al. [58] developed nano-sized ice (NI) slurry with ice crystal size 400–700 nm, which is more like a gel in nature rather than crystalline. The use of NI further enhanced the advantages of the effects of SI as mentioned earlier. In addition, SI is known to separate and form a hard ice crust layer, and it often requires breaking with shovels and results in damage to fish. The gel-like nature of NI and reduced buoyancy combine to prevent ice crusts from forming. However, more rapid melting could be an issue in chilling or achieving temperature threshold. Therefore, a proper ice–fish ratio and best handling practice with the melted water need to be used.

Most fish are chilled with ice soon after harvest unless they are destined only to nearby local markets. Researchers have demonstrated that the importance of rapid chilling cannot be over-emphasized [11]. Chilling reduces the rate of chemical and biochemical changes as well as microbial growth. The temperatures at which fish and seafood materials live are relatively low; thus chilling does not have as great an effect as the chilling of meat from warm-blooded animals [11]. Ice is a very good coolant. It is also cheap, and it must be made from clean, unpolluted, and bacteria-free water. Chilling generally reduces the temperature of fish to 0°C [11]. Chilling to 0°C in ice or refrigerated compartments is an essential requirement for quality retention in most fish and seafood products. For a longer storage period, fish and seafood may also be frozen. From a microbial standpoint, storage at –2 to –4°C is better than at 0°C or 2–5°C; however, this is not necessarily the case from a quality perspective due to toughening and autolytic changes which occur at the lower temperatures such as lipid hydrolysis. Shelf life may be extended further by using sorbate or sulfite [15]. The shelf life of selected types of raw fish as a function of temperature is shown in Table 8.8.

In experiments with fish during ice storage, the penetration of microorganisms occurred primarily from the intestines, the skin flora being found in the flesh only during the later stages of spoilage (8–17 days depending on species) [62, 63]. It was also observed that the quality and keeping time of the trout was reduced when the fish was exposed to physical stress. The infection level in the fish muscle increased with increasing physical stress and was higher for feeding than for starving trout [64]. When gutting the fish, it was observed that the intestines appeared pale and bloodless in the treated samples. This could be the result of a stress condition, which increased the production of adrenalin, thus forcing the blood into muscles and gills. There was good correlation between the log count and the organoleptic score when the bacteria count was higher than 100/g. The lack of correlation at lower bacterial counts may be expected since autolytic spoilage processes are more active in the earlier storage period and it is the dominant factor influencing organoleptic assessment [64].

Black carp fillets could maintain good sensory acceptability for 2, 9, and 15 days during storage at 20°C, 4°C, and 0°C, respectively, while the combined effects of K and TVB-N values showed good quality for 2, 9, and 12 days [65]. *Pseudomonads*, *Aeromonas*, and *Enterobacteriaceae* were the main spoilage bacteria in black carp. Tyramine and putrescine were the main biogenic amines while tryptamine, 2-phenylethylamine, putrescine, cadaverine, and tyramine increased during storage at all storage temperatures. Carp stored at 20°C favored the formation of histamine during storage time and the concentration of histamine was 132.1 mg/kg on the third day, while histamine concentration was 0.62–3.28 mg/kg (during storage period) while stored at 4°C and 0°C. Tyramine, cadaverine, and histamine were highly correlated with the development of tyrosine, lysine, and histidine, respectively, and electrical conductivity (EC) was correlated with sensory, physical, chemical, and microbial parameters. However, EC could be used as a better quality indicator to assess the overall quality of fish stored at 4°C and 0°C as compared to that at 20°C.

TABLE 8.8
Approximate Shelf Life of Selected Types of Raw Fish at Different Temperatures

Fish	Temperature (°C)	Shelf Life (Days)
Cod	0	16
	5	7
	10	4
	16	1
Herring	0	10
	5	4
Salmon	0	2
	10	5
Plaice	0	18
	10	8

Source: [11].

8.3.4 Chemical Treatments and Use of Bio-Preservatives

A wide range of chemical treatments and living cultures of bacteria have been used in commercial practice or proposed by researchers for mitigating the deleterious effects of abiotic, biotic, and physiological factors, which cause spoilage in fish and seafood. The principal chemical agents used are chlorine and hydrogen peroxide.

8.3.4.1 Chlorine and Chlorine Dioxide

Lin et al. [66] found that a commercial chlorine dioxide killed *Escherichia coli*, *Listeria monocytogenes*, and its streptomycin-resistant strain at 15, 10, and 7.5 ppm. The authors also reported that aqueous chlorine dioxide was more effective than aqueous chlorine in killing *L. monocytogenes* on fish cubes and in washed-off solutions. Fish cubes treated with aqueous chlorine showed no visual changes in color. Treated solutions became lightly milky. A light brown color occurred on fish cubes treated with aqueous chlorine dioxide at 400 ppm. The treated solutions had a light pink (40 and 100 ppm)

to light yellow (200 and 400 ppm) color, with some turbidity. The fish cubes treated with aqueous chlorine or chlorine dioxide contained no detectable chlorine residues, but commercial chlorine dioxide solution showed chlorite and some free and combined chlorine, especially at 200 and 400 ppm.

8.3.4.2 Hydrogen Peroxide

Dipping in a hydrogen peroxide solution can increase the shelf life of fish. Hydrogen peroxide acts as a preservative as well as a bleaching agent, thus yielding a higher quality product with an extended shelf life. A major point of concern had been that hydrogen peroxide treatments could lead to excessive oxidative rancidity, thereby causing a marked decrease in the overall quality of product [67]. Sims et al. [67] reported that hydrogen peroxide was entirely dissipated in the flesh within 0.5 hours when raw herring were immersed in dip solutions containing up to 600 ppm hydrogen peroxide. The color observations carried out on samples from the treatments indicated that the fillets with hydrogen peroxide were considerably whiter than untreated fillets.

8.3.4.3 Lactic Acid Bacteria

The addition of living cultures of lactic acid bacteria is used to control pathogen growth in fish. *Listeria monocytogenes* is difficult to control in lightly salted conditions (<6% NaCl in aqueous phase) with pH above 5, and at storage temperatures around 5°C. Wessels and Huss [68] noted that *L. monocytogenes* in lightly preserved fish products can be controlled using food-grade lactic bacteria. The effect was not due to lactic acid inhibition, but to the production of the natural preservative nisin by the lactic acid bacteria. Sodium chloride solution up to 4% allowed for efficient growth and nisin production, while 5% sodium chloride resulted in very slow growth and no detectable nisin.

8.3.5 Enzyme Inhibitors

The tenderization or flesh softening of seafood has generally been attributed to the activity of endogenous muscle proteases in the postmortem animal. The undesirable postmortem activities of these enzymes have been controlled by low-temperature and chemical treatments [69]. The use of plasma glycoprotein and α2-macroglobulin (the active component in egg white plasma hydrolysates) can be used to inhibit several endogenous proteases [70]. α_2-Macroglobulin noncompetitively inhibited the proteases, in decreasing order: cathepsin D > trypsin > chymotrypsin > collagenase. The inhibitor's activity depended on the size of the substrate molecule, the size of the enzyme, and the relative specificity of the enzyme. Thus, it could control desirable proteolytic activities. In intact fish and other muscle foods, it may be restricted to tissue penetration due to membrane barriers and the relatively large molecular size of the inhibitor. Proteases in surimi and other minced muscle foods should be readily inhibited by it. The activity did not seem to be adversely affected by low temperatures (4–7°C) [69].

Prawns develop blackspot (melanosis) in chilled and frozen storage, and sulfite is a reducing agent used to prevent this discoloration. The presence of sulfite reverses the formation of colored compounds (quinones) and in addition acts as a competitive inhibitor of polyphenol oxidase, the enzyme that causes the production of the pigment melanin [71, 72]. Recently, it was reported that 4-hexylresorcinol binds irreversibly to polyphenol oxidase, inhibiting its action [73]. The likelihood of adverse reactions in humans from the low levels found in prawns was considered slight [74]. Sensory panel assessment found no effect on the taste, texture, visual appearance, and development of normal colors after cooking by treating prawns with 4-hexylresorcinol. Slattery et al. [74] used Everfresh® (which contains 4-hexylresorcinol) to inhibit polyphenol oxidase in trawled and farmed prawns. In comparison with sodium metabisulfite treatment, Everfresh (0.2% 4-hexylresorcinol) provided greater protection against blackspot, particularly on the body of the prawn during storage in ice, in refrigerated seawater, and in ice after frozen storage. When Everfresh was used according to the manufacturer's recommendations, residues of 4-hexylresorcinol were less than 2 μg/g in prawn flesh.

8.3.6 Super Chilling

Super chilling storage incorporated by an ice glazing (SS-IG) approach could minimize fat rancidity, fluid loss, and microstructure disarrangement [75]. The traditional material used as IG layer is water. A thin layer of IG is formed by dipping fish into an icy solution bath to produce a protective barrier on the surface, and it could exclude air and reduce the effects of temperature fluctuations [76]. In addition, extracts (such as essential oil and tangerine peel) from natural plants were shown to be an effective alternative [77–79]. Essential oil of 0.1, 0.2, and 0.3% v/v solutions were used to immerse fresh sea bass samples at ~0°C to form IG layers [75]. Fish was stored at –1°C for 25 days, and a series of freshness assays showed that the treatment was effective in maintaining the storage freshness by retarding the spoilage of preserved fish samples in electric, chemical, microbial, textural, and sensory properties. The SS-IG approach could be promising in the aquaculture industry.

The quality of iced, chilled (+1°C), and super-chilled (–1°C) cod was evaluated by microbial analysis, total volatile base nitrogen (TVB-N), and sensory evaluation [80]. The H_2S-producing bacteria appeared to grow more rapidly in the fish that was iced whole as compared to those that were super-chilled on-board. Total viable counts in fillets were lower during super-chilled storage conditions. The TVB-N level of fish iced on-board and stored chilled rose above the consumption limit on day 11 after catch, while the levels in super-chilled fish remained below the permitted limit. TVB-N levels where fillets were stored super-chilled were still under the limit after 16 days although sensory evaluation rejected both groups. Considering sensory quality, super-chilled storage of fillets showed a much greater impact, resulting in 2–4 days extension of freshness and 3 days longer shelf life. The effects of on-board whole fish super-chilling were less noticeable except for the growth of H_2S-producing bacteria and spoilage attributes (TVB-N, decomposition of proteins, and sensory).

8.3.7 Irradiation Treatment

Gamma radiation is considered an innovative and interesting method to preserve chilled, stored fish and to reduce microbial populations in fresh fish and fish products [81–85]. Several researchers have reported increases in storage times of 1–3 weeks for fresh and cooked product and doubling of storage times for frozen products [86]. Several authors [87, 88] have noted that irradiation doses of 2–7 kGy can reduce important pathogens in food such as *Salmonella*, *Listeria*, and *Vibrio* spp., including many fish-specific pathogens like *Pseudomonadaceae* and *Enterobacteriaseae*, which can be significantly reduced in number.

The application of irradiation treatment in fish and seafood must be viewed as part of integrated quality and safety management, incorporating good manufacturing practice (GMP) and hazard analysis and critical control points (HACCP). Thus, the fish destined for irradiation must be in good quality, free from defects, and handled properly prior to and after irradiation. It is only under these conditions, with good irradiation practices, that most pathogenic microorganisms can be eliminated, and spoilage bacteria can be sufficiently reduced so that significant increases in storage time can be achieved.

Irradiation doses between 0.75 and 1.5 kGy for fresh products and cooked products and between 2 and 5 kGy for frozen foods have been recommended [86]. Marcotte did not consider these doses could be sufficient in controlling spore-forming bacteria such as *C. botulinum* Type E. Furthermore, it was noted that irradiation does not eliminate the toxins produced by *S. aureus* and others, and consequently, the author cautioned that, whether irradiated or not, fish and shellfish must be properly processed and stored cold (<3°C) or in ice, or frozen. Other researchers have reported that fish may be irradiated at doses of 3–4 kGy, without an appreciable increase in temperature during irradiation [85], and without affecting odor and taste, thus increasing the storage life of the product by two- to three-fold [82].

In a recent study to determine the effect of 60 Co gamma irradiation on Atlantic horse mackerel—a highly perishable fish species abundant off the coasts of Portugal and Spain and an important component of the diet in these countries—Mendes et al. [84] applied two levels of irradiation (1 and 3 kGy). Irradiated and uneradicated (control) fish were stored in ice for 23 days after which a series of chemical tests of spoilage were carried out. The authors found that a dose of 1 kGy appeared adequate to extend the shelf life of the fish by 4 days, and increasing the dose to 3 kGy did not give appreciable additional benefits in terms of quality attributes. No detrimental effect on the quality of fish by irradiation was evident.

8.3.8 High-Pressure Treatment

The freshness of fish is caused primarily by endogenous enzymes and microorganisms present in fish flesh [89]. High-pressure processing of fresh fish could be used as one of the thermal treatments [90]. This pretreatment was used in the case of hilsa fillets [91] and sea bass [92] during refrigerated storage; fresh salmon, cod, and mackerel [93], and frozen stored hake [94] and mackerel [95]. Tilapia fillets were treated with high-pressure processing (i.e., 100–400 MPa for 1 or 3 min), and quality attributes during refrigerated storage were evaluated [96]. Color, texture, sensory, and microbiological analysis showed that high pressure of 400 MPa for 3 min was efficient in preserving the fillets in refrigerated storage (5°C) for 1 week. The appearance due to change in color could be an issue for commercialization although fish preservation is achieved.

8.3.9 Edible Coating

Bio-based coating is considered an effective and eco-friendly choice for maintaining the freshness of food products and extending their shelf lives [97]. Chitosan coating combined with glycerol monolaurate was used to extend the shelf life of refrigerated grass carp [97]. Gelatin is widely used in preparing edible coatings and films, but it has poor preservation characteristics, such as antioxidant and antimicrobial abilities [98]. Curcumin possesses potent antioxidant and antibacterial properties and could be used in the coating. Edible coating combining fish gelatin with curcumin-β-cyclodextrin (CUR-βCD) emulsion was effective in maintaining the quality and extending the shelf life of grass carp fillets during storage at 4°C [99]. The coatings containing CUR-βCD emulsions exhibited better preservative effects than gelatin-βCD coating. The quality parameters included were: weight loss, pH, total volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid (TBA) value, SDS-PAGE, free amino acids (FAA), microbiological (total viable counts (TVC), *Pseudomonas* counts, yeasts and molds, and H_2S-producing bacteria), color, and sensorial characteristics.

8.3.10 Role of Packaging Technology

Proper packaging plays a crucial role in the preservation of quality and delivery of safe, wholesome fish and seafood products to the end-user. Packaging performs three main functions, namely containment, protection, and information. With regard to fresh fish and seafood, packaging must be carefully selected to cope with the presence of water on fish skin, a condition that could contribute to the breakdown of paper-based packaging as well as rapid microbial contamination. Packaging must also be selected to protect against adverse environmental and atmospheric conditions as well penetration of physical and chemical hazards. To facilitate supply chain management and marketing operations, adequate labeling of the package is essential to inform and educate the end-user about the content and utility. Given the high perishability of fish and seafood, only blemish-free and top-quality produce should be contained in the package.

Innovative packaging technologies based on manipulating the gas exchange characteristics of packaging material to control the redox potential have been developed and applied to preserve and extend the storage stability of fish and seafood products. The application of vacuum packaging, controlled

(CA) or modified atmosphere (MA) packaging around fresh fish is based on the following premise: some spoilage bacteria and lipid oxidation require oxygen—thus, reducing the oxygen around the fish will increase storage and shelf life. Depending on the fish species and intended end-use, specific combinations of O_2, CO_2, and N_2 determine the level of CA or MA. In practice, vacuum packaging, CA storage, and MA packaging are used in combination with refrigerated storage for preservation of fish and seafood products. The combination of methods must be optimized and closely evaluated to match specific requirements.

The use of biodegradable green polymers for packaging from renewable sources is highly desirable for food preservation and packaging. Polylactic acid (PLA) is one of these naturally originated polymers. The polymer composition and addition of nanoparticles (i.e., nano-silica and organomodified montmorillonite) could adjust the release of preservatives from the polymeric material [100]. Active packaging films based on PLA containing 1.5% w/w zinc oxide (ZnO) nanoparticles and varying concentrations (0.5, 1, 1.5% w/w) of essential oil (EO) were used to preserve the refrigerated fish fillets [101]. The effects of the active films on the shelf life extension of fish over 16 days of refrigerated storage (4°C) showed the extension of shelf life from 8 to 16 days. The shelf life was determined considering microbial count (aerobic count, *Enterobacteriaceae count*, *Pseudomonas spp*., lactic acid bacteria, and H_2S-producing bacteria) and chemical levels (TBARS and TVB-N). Carvacrol and menthone were found to be the main antibacterial compounds.

8.4 CONCLUSION

Fish and other marine products are important sources of food throughout the world and in particular for over 1 billion people as their main source of protein. Global consumption of fish is rising steadily and has doubled since 1973 [33]. Current estimates project that the demand for fish will continue due to increasing population growth and increasing purchase because of further realization of the health and nutritional benefits of fish in the diet. However, serious concerns have been raised about the ecological effects of industrialized fishing and the resultant rapid worldwide depletion of predatory fish communities [102]. This scenario has propelled aquaculture (the farming of fish, shellfish, and seaweeds) as the fastest growing sector in global food production.

Despite the growing demand and importance of fish and seafood in the human food system, the incidence of postharvest losses, quantitatively and qualitatively, remains high, especially among many rural fishing communities. Reducing fish losses and preserving fish quality over extended storage periods will contribute towards improved food security and income without the need for additional catches to meet growing demand. Unlike the situation for red meat and poultry, the fact that the majority of food fish and seafood are "hunted" in the oceans and seas presents additional technical challenges to adequately control quality deterioration and preserve the produce. Thus, improved harvesting techniques and procedures are needed which reduce the lag time between catch and landing to avoid unacceptable losses in quality (and quantity) prior to handling and processing.

High consumer preference for fish in fresh form (as opposed to processed product) assures future demand for innovative treatments and preservation techniques that maintain freshness and optimum eating quality over an extended period of time. Continuing research is also needed to develop low-cost, portable, non-destructive devices for rapid measurement of freshness. Such a device will be useful for research as well as use by quality control personnel responsible for enforcing relevant regulations and directives on fish quality standards and marketing.

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9 Postharvest Storage and Safety of Meat

Isam T. Kadim, Quazi Mohd. Imranul Haq, Issa S. Al-Amri, Abdulaziz Y. Al-Kindi, and Amera K. Nasser

CONTENTS

9.1 INTRODUCTION

Meat is the edible part of the flesh of an animal's skeletal muscles and is an excellent source of many nutrients for human consumption. The term "postharvest fresh meat" includes meat from recently processed animals as well as vacuum-packed meat or meat packed in controlled-atmospheric conditions to ensure quality and preservation. However, the diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage microorganisms and common foodborne pathogens. It is essential that adequate preservation technologies should be applied to maintain its safety and quality [1]. The postharvest processes used in meat preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation are sought to minimize quality deteriorative changes such as color and oxidative changes.

The bacterial growth that causes meat spoilage is influenced by several factors, including intrinsic factors (physical and chemical properties, e.g. water activity, structure, content of nutrients), extrinsic factors (e.g. the composition of the atmosphere, storage temperature), processing factors (chemical and physical methods used during processing, e.g. cooking), and implicit factors (reflection of the antagonistic and synergistic effects between bacteria) [2]. A number of interrelated factors influence the shelf life and quality of postharvest meat including temperature, oxygen, endogenous enzymes, moisture, light, and microorganisms [3].

Several articles have reported the dominance of different pathogens in meat; *Campylobacter* (Figure 9.1) and *Salmonella* (Figure 9.2) are highest among them [4]. Meat-contaminating bacterial pathogens from the gastrointestinal tract are *Salmonella enteric* strains, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Escherichia coli* (Figure 9.3), and *Listeria monocytogenes* (Figure 9.4) [5]. For meat poisoning outbreaks, poultry meat is highest, and it is an ideal, very well-suited medium for bacterial culture [5].

Although fresh meat quality deterioration can occur due to proteolysis, lipolysis, and oxidation, microbial growth is the most important factor affecting the quality of fresh meat [6]. Poisoning resulting from the ingestion of toxins, exotoxins (secreted by bacteria such as *Clostridium botulinum* (Figure 9.5), and endotoxins (released upon the death of microorganisms) [7]. Postharvest meat preservation can be prolonged

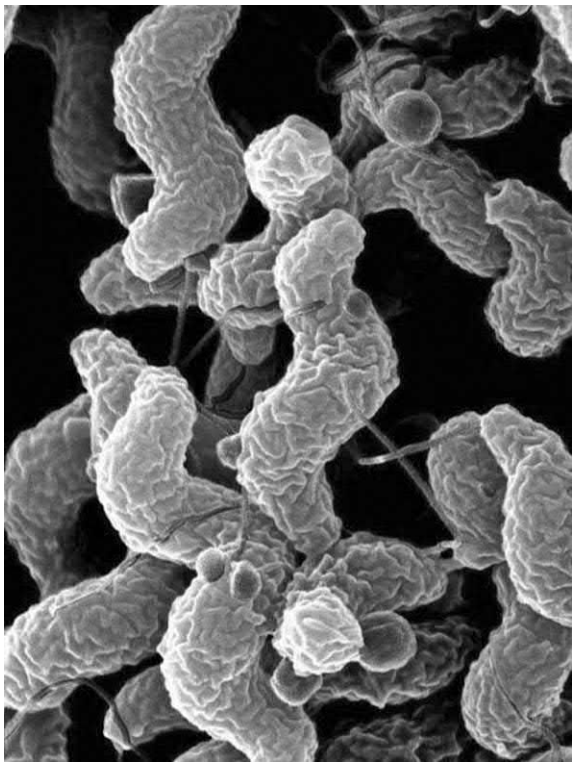

FIGURE 9.1 Morphology of *Campylobacter jejuni*. (From http://commons.wikimedia.org/wiki/file:ars_ campylobacter_jejuni.jpg.)

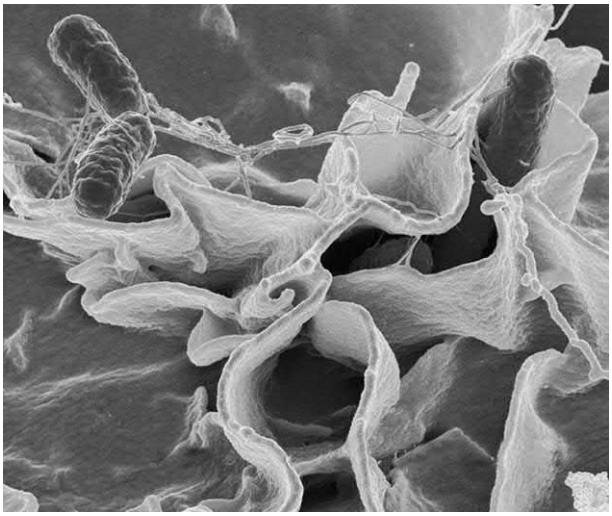

FIGURE 9.2 Morphology of *Salmonella*. (From Wikipedia. Credit: Rocky Mountain Laboratories, NIAID, NIH. http://en.wikipedia.org/wiki/Bacteria.)

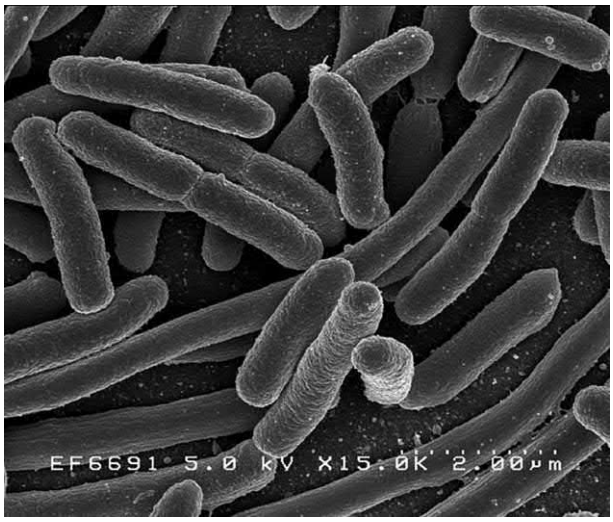

FIGURE 9.3 Morphology of *Escherichia coli*. (From Wikipedia. Credit: Rocky Mountain Laboratories. http://en.wikipedia.org/wiki/Bacteria.)

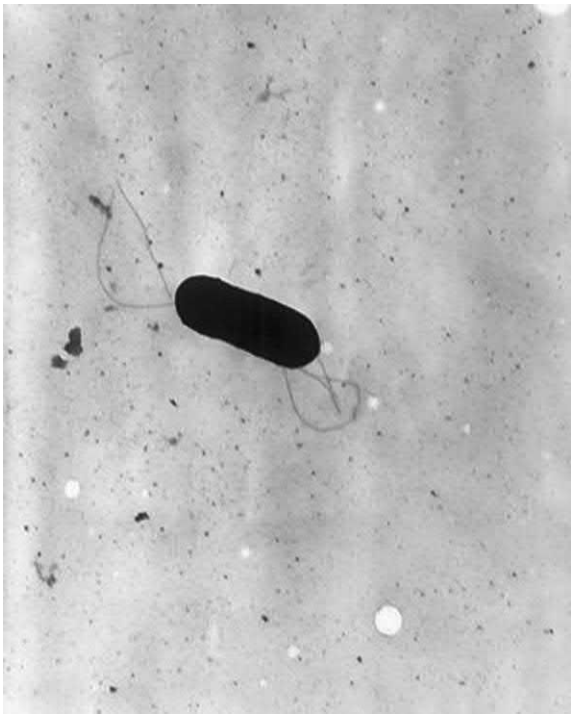

FIGURE 9.4 Morphology of *Listeria monocytogenes*. (From CDC. http://phil.cdc.gov/phil/home.asp.)

by controlling the atmospheric temperature, moisture, and inhibitory processes of microbial proliferation. New preservation technologies for fresh meat preservation include nonthermal inactivation such as modified atmosphere packaging, active packaging, natural antimicrobial compounds, and biopreservation. The future for fresh meat products will depend mainly on consumer demand and the prices at which they can be profitably produced. Therefore, fresh meat requires proper handling after harvesting. Enhanced meat safety involves the application of measures to delay or prevent microbiological, chemical, and/or physical changes that make meat healthy for human consumption. Bacterial foodborne infections are increasing at a very high rate worldwide [8]. The consumption of raw meat contaminated with multidrug-resistant (MDR) *Salmonella* is a global public health crisis; annually, more than one billion humans are infected, suffering from gastroenteritis, and around five million individuals die [9]. Another

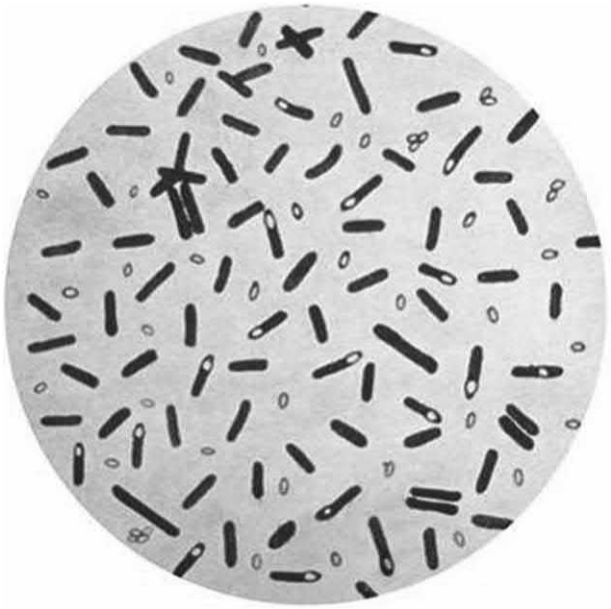

FIGURE 9.5 Morphology of *Clostridium botulinum*. (From CDC. http://phil.cdc.ov/phil/home.asp.)

multidrug-resistant bacteria, *Listeria* spp., is reported in raw meat and meat-related products from Malaysia [10]. Beef meat contamination with *Clostridium difficile*, which is a leading cause of pseudomembranous colitis and nosocomial diarrhea, has been confirmed recently [11]. Resistant bacterial strains that are resistant to many antibiotics are transmitted to humans from animals through uncooked meat and contact with meat surfaces [12]. This chapter aims to review the technologies for the preservation of fresh meat and enhancement of the quality characteristics.

9.2 MUSCLE STRUCTURE

The shelf life of fresh meat depends on the microflora present on the meat, which creates an ecosystem that is affected by many factors, such as the intrinsic properties of the meat, the initial microbial contamination load, temperature, storage time, and the processing method [7]. Fresh meat bacterial contaminants mainly include the genera of *Pseudomonas*, *Acinetobacter*, *Brochothrix*, *Psychrobacter*, *Flavobacterium*, *Micrococcus*, *Staphylococcus* (Figure 9.6), *Moraxella*, various genera of the family Enterobacteriaceae, and lactic acid bacteria [7]. Skeletal muscle is built up of thousands of cylindrical muscle fibers often running all the way from origin to insertion. They are forced together by endomysium connective tissue through which run blood vessels and nerves (Figure 9.7). The conventional instruction for understanding red meat is to start with the muscle structure. Muscle biology is complex due to its various biological functions, such as its role in contraction, accumulation of protein, and protection [13]. Muscle metabolism plays a role in the pathogenesis of metabolic disorders and in the transformation of muscles to meat [14]. Animal physiology also generally plays an important role in controlling the changes that occur in the postmortem conversion of muscle to meat, thereby affecting meat quality [15, 16]. Muscle characteristics are of prime importance since quality is recognized as one of most important social and economic challenges for meat producers and retailers around the world.

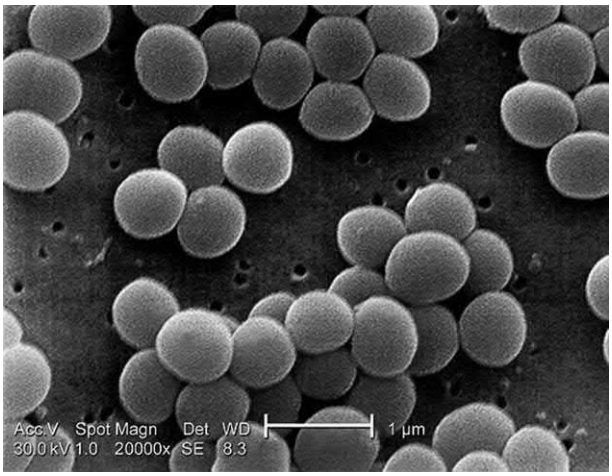

FIGURE 9.6 Morphology of *Staphylococcus aureus*. (From CDC. http://phil.cdc.gov/phil/home.asp.)

Many of the biochemical reactions present retain some degree of activity in animal muscles after death. The biochemical reactions are responsible for quality changes during storage. The rate and extent of muscle postmortem metabolism are dependent on the availability of glycogen at slaughter [17], the temperature of the medium in which the reactions occur [18], and whether or not procedures intended to accelerate metabolic reactions have been applied [19]. Initially, muscles become stiff and hard but gain some softness after hanging and aging.

9.3 CONTAMINATION OF HARVESTED MEAT

There are two approaches to inactivate microorganisms under chilling conditions; the first one is to change the environmental conditions, and the other is to decrease the tolerance of the microorganisms. In the meat sterilization process, the inactivation of dormant bacterial spores is the main objective. These spores are highly resistant to heat, drying, radiation, and chemicals. As germinated spores are not resistant to these agents, it is effective to germinate dormant spores and then inactivate them. Many of the microorganisms that influence meat spoilage require the presence of oxygen to grow. Vacuum-packed meat is also preserved from weight loss and discoloration. Sanitation programs throughout the chilling and storage operations can maximize the shelf life of fresh meat cuts. Reducing the initial microbial contamination of meat significantly decreases the influence of microbes in changing meat odor, appearance, and flavor. Vacuum-wrapped cuts, at the proper temperatures, may be displayed for extended periods during marketing without the occurrence of foodborne pathogens.

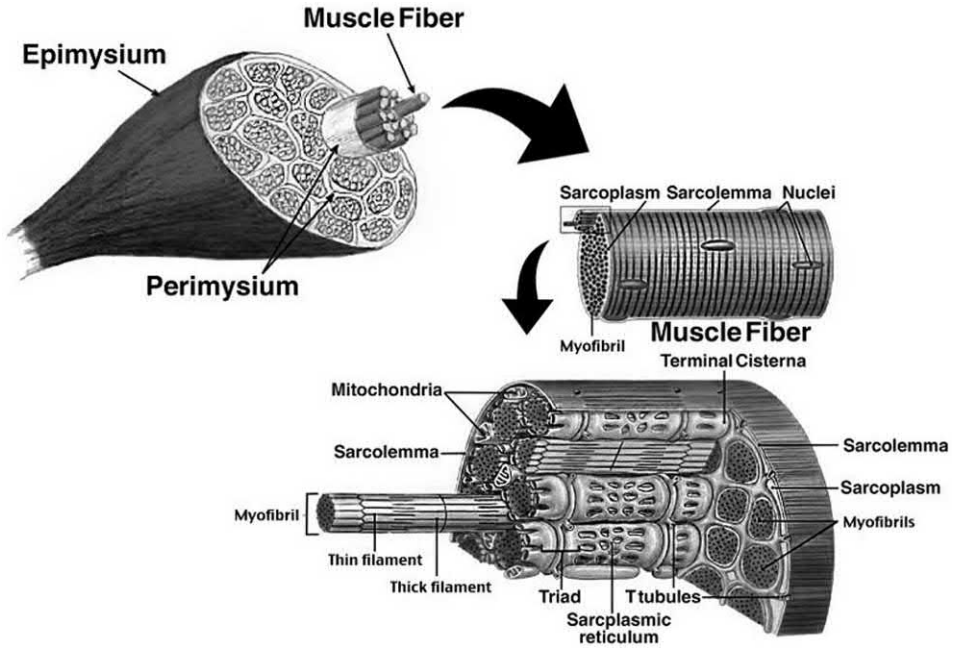

FIGURE 9.7 Microstructure of animal muscle.

9.3.1 Fresh Meat Processing

During meat storage, bacterial metabolic activity may lead to meat spoilage. The methods of operational hygiene for meat handling and packaging are responsible for the composition of the different microflora that develops on meat [2]. Contamination of fresh meat is a direct consequence of processing [20]. By the application of antimicrobial films, the inhibition of surface spoilage bacteria is studied [21]. Many microorganisms from various sources are introduced to nutrient-rich fresh meat surfaces. Although a smaller proportion of microorganisms will predominate and cause spoilage, only 10% is capable of survival and proliferation during storage, distribution, and retail sales of meat at low temperatures. A successful hygiene process extends meat shelf life and allows its delivery from processor to consumption areas. It is generally recognized that the most significant foodborne hazards from fresh meat are bacterial pathogens, which cause many human diseases, such as *Salmonella*, *Campylobacter*, and pathogenic *E. coli* (Figure 9.8) such as *E. coli* O157. Some of these, particularly *E. coli* O157, require only a few bacterial cells to cause food poisoning in humans. The safety of meat and meat products is important to producers, retailers, and consumers. There are mainly two types of microorganisms (i.e. pathogenic and non-pathogenic) able to multiply in meat during chilled storage conditions. The preservation of meat depends on the number of spoilage microorganisms present initially, the temperature history of the meat at all stages of processing, subsequent storage, and handling practices. The problem may be due to several stages in meat processing, in which meat remains in close proximity throughout the operation. Such conditions favor the spread of any pathogens that may gain access to the facilities.

9.3.2 Control Contamination

Fresh meat should be entirely safe from microorganisms; therefore it should be free from all pathogenic organisms. However, practically this is not a realistic goal for fresh meat production. Therefore, some level of product contamination must be tolerated, although this varies widely from one country to another. Control of meat's microorganisms is difficult and subject to cost constraints including implementation of hazard analysis and critical control points (HACCP) in meat-processing plants. However, improved practices in production and processing plants may lead to a steady decline

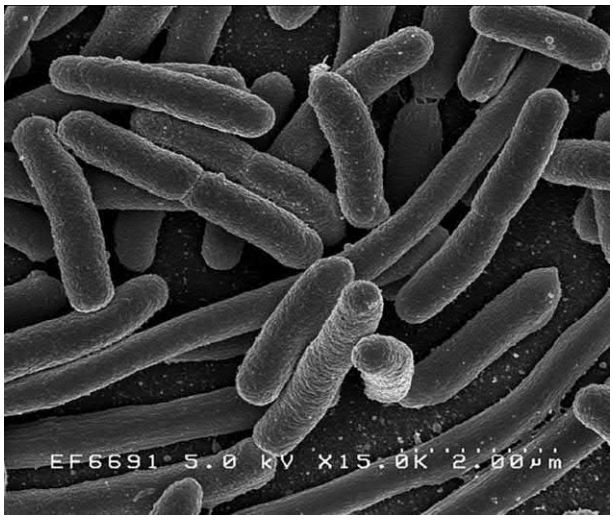

FIGURE 9.8 Morphology of *Escherichia coli*. (From Wikipedia. Credit: Rocky Mountain Laboratories. http://en.wikipedia.org/wiki/Bacteria.)

in the contamination rate [22]. Regular rejection of contaminated fresh meat would be economically unacceptable on the scale required. Instead, there is a growing emphasis on the application of preventative measures within the meat industry, and there is now much reliance on the HACCP system for controlling foodborne pathogens in meat-processing plants. The microbiological hazards in the processing operation are well-known and are difficult to control effectively, because of the technological limitations in the process which can lead to cross-contamination of the meat being processed. Implementation of the HACCP system does not overcome this drawback but has a number of clear benefits, including that the system ensures regular monitoring of the process, hygiene control is optimized, control parameters are an integral part of the system, compliance with hygiene legislation is ensured, and staff awareness of food-safety is necessary. Cross-contamination of fresh meat with pathogens can occur at every stage of the process, and there is little evidence that this problem is significantly reduced by the application of HACCP principles. Moreover, there is no critical control point at which a significant reduction in pathogen contamination can be guaranteed. Without the use of processing aids to improve hygiene, the greatest reductions in fresh meat contamination are likely to come from technological developments in the process that are designed to improve hygiene, as long as these are acceptable to the industry.

9.3.3 Decontamination

The grouping concern about the contamination of fresh meat with enteric pathogens has led to extensive investigation of treatments for reducing the number of bacteria on fresh meat products. Decontamination is very important in reducing meat spoilage and consequently improving meat hygiene. Both psychrotrophic and mesophilic bacteria can grow at temperatures above 20°C [23]. Short-term chilled storage at less than 10°C is the safest procedure for storing meat products. For long-term storage, the temperature should be as near to the freezing point as practicable (1.5°C), and relative humidity should be controlled within 85–95% to prevent drying or condensation on the meat surface. The main spoilage microorganisms present in aerobic conditions in low-temperature storage are *Pseudomonas* species, whereas under vacuum storage, the major spoilage microorganisms are *Lactobacillus* species and yeasts [24]. Reducing fresh meat surface contamination and avoiding or limiting microbial growth would improve safety and external shelf life [25]. Removal of bacteria stuck on the fresh meat surface, followed by spraying antimicrobial hot water, induces the release and inactivation of bacteria, which is necessary for effective decontamination [26]. The possible heat damaging of the appearance of the fresh meat surface reduces the feasibility of such treatment. Steam is one of the more effective and fast methods for reducing the number of pathogenic bacteria on meat. The surface will appear quite rough with many pores. It is difficult to kill bacteria that get into these pores with sanitizing solution because surface tension prevents the liquid from entering the pores. Therefore, steam should be able to enter the pores and kill the bacteria.

9.3.4 Organic Antimicrobial

Surface treatment with organic acids is a more realistic option for eliminating pathogens without adverse effects on the quality of the meat. The use of organic acids reduces bacterial counts in the meat surface layer; lactic acid is often used, as it is a natural meat compound produced during postmortem glycolysis [27]. The combination of physical treatment with hot steam and spraying with a lactic acid solution is another approach for fresh meat surface decontamination [28, 29]. Acid washes have been shown to be effective in reducing the total number of microorganisms present on meat [30]. The antimicrobial effect of the organic acids is due to the reduction of pH below the growth range and metabolic inhibition by the un-dissociated molecules [31]. In this respect, acid and heat inactivation of microorganisms follows the release of microorganisms from the surface. Moreover, the lactate anion slows down the growth of surviving microbes during storage [26]. The antimicrobial action of organic acids depends on three factors: (i) pH, (ii) extent of dissociation, and (iii) specific effects related to acid molecules. The effective growth inhibition by an acid only occurs when an appropriate amount of the un-dissociated molecule is present [32]. This amount may be obtained by either applying more acid or by lowering the pH. Most organic acids are therefore effective only at low pH values, i.e. below ~ pH 5.5. The differences in antimicrobial activities of various organic acids are related to (i) the potency to penetrate a cell, (ii) the part of the cell which is attacked, and (iii) the chemical nature of that attack [32]. The factors influencing the efficacy of acids treatments are (i) nature of meat surface and initial level of contamination, (ii) initial bacterial load, (iii) type of acid used, (iv) concentration and temperature of the acid, (v) types of microorganisms present on the surface [33]. After examining 13 acids, it was recommended that acetic and propionic acids are the most effective agents for microorganisms [34]. Mixtures containing various acids may also be used for their synergistic effects on microorganisms, but synergistic effects may not always be observed. Acetic and lactic acids (1–4% concentration) produce the desired microbial reduction, and their mixture may enhance the effectiveness [33].

Decontamination systems may adversely affect meat quality characteristics that contribute to meat acceptability, such as color, flavor, odor, and drip loss. An efficient decontamination system should reduce bacterial numbers without any detrimental changes to the quality parameters [28]. In general, treatment with lactic acid, acetic acid, and citric acid at low concentrations does not produce much of an adverse effect on color [28]. However, higher concentrations resulted in bleaching of lean meat and fat [33]. When many blood spots are present on the meat surface, the coagulation of blood may cause rusty brown-black spots. This is more particularly evident at higher acid concentrations, but decontamination with acetic or lactic acids at low concentrations of 1 to 2% hardly

affects the sensory quality of meat [33]. The sensory scores are more readily affected by acetic acid than lactic acid; thus mixtures may help to alleviate color problems. Slight visible discoloration forms immediately after application at low concentrations, and usually disappears upon diffusion of the acid. The use of acetic, propionic, lactic, and formic acids for decontamination is considered to be acceptable from a toxicological perspective [35].

Natural compounds including essential oils, chitosan, nisin, and lysozyme, have been replaced by other materials to preserve meat and meat products. The use of natural or lactic acid bacteria and their antimicrobial products including lactic acid and bacteriocins to extend the storage time and safety of meat has been investigated [36]. Bacteriocins are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin, and biochemical properties, which can be used for meat preservation [37]. Eugenol in cloves and allyl isothiocyanate in mustard seed have preservative properties, and these have been used to extend the storage period of meat products. Nisin is the commercial bacteriocin and has been used to decontaminate artificially contaminated pieces of raw meat [38] and in combination with 2% of sodium chloride as an anti-listerial agent in minced raw meat [39]. The bacteriocins produced by lactic acid bacteria are listed in Table 9.1.

Pentocin produced by *Lactobacillus pentosus* and isolated from fermented meat has been used as a biopreservative in storage-chilled meat. Results showed that pentocin could substantially inhibit the accumulation of volatile basic nitrogen and suppress the growth of microflora, especially *Listeria* and *Pseudomonas* [40].

9.3.5 Inorganic Antimicrobial

Inorganic phosphates, hydrogen peroxide, and ozone can be used for meat decontamination. Trisodium phosphate treatment is officially accepted and widely implemented in the fresh meat process [41], and it does not cause undesirable sensory effects detectable by the consumer [42]. A concentration of 10–12% in alkaline solution could be used [41]. The formation of radicals by hydrogen peroxide damages nucleic acids, proteins, and lipids, thus causing a bactericidal-bacteriostatic effect [83]. Hydrogen peroxide was used as a meat decontaminant at a minimum effective dose of 0.5% (v/v) in water. At this level, a temporary bleaching and bloating of the fresh meat [43] and excessive foaming of chiller water are observed [41], and the application of hydrogen peroxide for decontamination seems to be an effective and safe method to control the spread of pathogens. Ozonated water is also used to eliminate bacteria counts on the fresh meat surface [44]. Ozonated water can be used to decontaminate meat products without visual defects or sensory off-flavors, although bacterial count reduction was poor (>1-log cycle) and there was no increase in shelf life [43]. Spraying beef meat with water followed by spraying with ozonated water is an effective bacteriological sanitation method [45].

TABLE 9.1
Bacteriocins Produced by Lactic Acid Bacteria

Producer Organism	Bacteriocin	Producer Organism	Bacteriocin
L. Lactis ssp. *lactis*	Nisin	*Lb. curvatus* LTH1174	Curvacin A
L. Lactis BB24	Nisin	*Lb. curvatus* CRL705	Lactocin 705
L. Lactis WNC	Nisin Z	*Lb. curvatus* FS47	Curvaticin FS47
L. lactis ssp. *lactis*	Lacticin 481	*Lb. curvatus* L442	Curvacin L442
L. lactis ssp. *cremoris*	Diplococcin	*Lb. plantarum* CTC305	Plantaricin A
L. lactis ssp. *lactis*	Lactostrepcins	*Lc. gelidum* UAL187	Leucocin A
L. lactis ssp. *diacetilactis*	Bacteriocin 550	*Lc. mesenteroides* TA33a	Leucocin A
L. fermenti 46	ND	*Lc. carnosum* TA11a	Leucocin A
L. helveticus 27	Lactocin 27	*P. acidilactici* L50	Pediocin L50
L. helveticus	Helveticin J	*P. pentosaceous* Z102	Pediocin PA-1
L. acidophilus	Lactacin B	*C. piscicola* LV17B	Carnobacteriocin B2
L. acidophilus	Lactacin F	*C. piscicola* V1	Piscicocin vla
L. plantarum	Plantaricin A	*C. piscicola* LV17A	Carnobacteriocin A
L. sakei Lb 706	Sakacin A	*C. piscicola* JG126	Piscicolin 126
L. sakei I151	Sakacin P	*C. piscicola* KLV17B	Carnobacteriocin B1/B2
L. sakei LTH673, 674	Sakacin K, P	*C. divergens* 750	Divergicin 750
L. sakei CTC494	Sakacin K	*C. divergens* LV13	Divergicin A
L. sakei L 45	Lactocin S	*E. faecium* CTC492	Enterocin B
L. sakei MN	Bavaricin MN	*E. faecium* CTC492	Enterocin A
Lb. brevis SB27	Brevicin 27	*E. casseliflavus* IM416K1	Enterocin 416K1
L. casei	Caseicin 80	*P. acidilacticii* PAC1.0	Pediocin PA1
P. acidilactic H	Pediocin AcH	*P. pentosaceus* FBB61	Pediocin A

Source: Stiles and Hastings [37].

Aqueous chlorine is also widely used in food processing to control microbial growth. Its bactericidal activity decreases in alkaline conditions and/or at high levels of organic matter. Furthermore, potentially toxic mutagenic reaction products, including trihalomethanes, are formed during the chlorine treatment of food components [46]. Chlorine dioxide has received much attention due to its advantages over aqueous chlorine: (i) it is seven times more potent than aqueous chlorine in killing bacteria [47], (ii) its bactericidal activity is not affected by alkaline conditions, and/or the presence of high levels of organic matter [48], (iii) it is less reactive than aqueous chlorine in interacting with organic compounds, such as unsaturated fatty acids, their methyl esters, and tryptophan and their derivatives [49].

It is possible to improve the hygiene levels of carcasses by means of antibiotics [50]. The most frequently used and cheap antibiotics are chlorotetracycline and oxytetracycline, and their combination is more effective [51]. However, the application of antibiotics to meat faces wide criticism and protest. Many people do not agree with antibiotic application to meat on legal and hygienic grounds as many antibiotics may cause toxic and allergic reactions as well as bacterial resistance with cumulative effects in the human body. Strict control of antibiotic applications in meat should be maintained [50]. In addition to the treatments, packaging is also used to prevent contamination, controlling the evaporation of water from the surface, and reabsorbing the drip. The absorbent and refrigerant pads benefit by absorbing and retaining unwanted fluids and improve the shelf life and help to maintain humidity levels.

Carbon dioxide and ozone have been used to prevent the growth of surface microorganisms on fresh meat during prolonged storage at chilled temperatures [36]. However, ozone can accelerate the oxidation of fat and is more effective against air-borne microorganisms than against those on meat surfaces [52]. Lactic acid is a frequently effective inhibitory agent used in fresh meat preservation, while other organic acids have been found to be responsible for discoloration and production of strong odors [53]. Sodium lactate has been used in the meat industry due to its ability to enhance meat flavor, prolong shelf life, and improve the microbiological safety of products [54–56]. Nadeem et al. [57] extended the shelf life of meat products stored at 5–7°C for 2–3 days, after spraying the meat with a solution containing potassium sorbate, sodium acetate, sodium citrate, and sodium lactate, each at 2.5%, and sodium chloride at 5%.

9.3.6 High Hydrostatic Pressure

Cheftel and Culioli [58] stated that high-pressure technology (100–1000 MPa, 1000–10,000 bar) is of increasing interest to biological and food systems. High hydrostatic pressure is of interest because it can inactivate meat product-spoiling microorganisms and enzymes at low temperatures without changing the sensory or nutritional properties of the meat. Pressure processing can be carried out in a steel cylinder containing a liquid pressure-transmitting medium (water), with the sample being protected from direct contact by the use of sealed flexible packaging. Maintaining the fresh meat under pressure at the chosen temperature for an extended period does not require any extra energy [58]. High hydrostatic pressure renders meat more stable due to its ability to reduce contamination with pathogenic microorganisms, and to inactivate certain meat enzymes [59, 60]. According to Hugas et al. [61], high hydrostatic pressure is a powerful tool to prevent the growth of *Salmonella* spp. and *Listeria monocytogenes* in raw or marinated meats.

The effectiveness of high hydrostatic pressure for microorganism control depends on the process parameters, pressure level, temperature, and exposure time, as well as on intrinsic factors of the meat itself, such as pH, strain, and growth stage of microorganisms, and meat composition [62]. It has been shown that the combination of high hydrostatic pressure with moderate temperatures can improve the tenderness of the meat [63]. However, high hydrostatic pressure at any temperature may have an undesirable effect on fresh meat color. The color of fresh meat [64] changes with pressure due to the denaturation of globin in myoglobin and heme displacement or release, and ferrous oxidation [65]. Denaturation of myosin and actin creates a greater opacity and therefore minimizes the red appearance.

9.3.7 Ionizing Radiation

Ionizing radiation has been a method of direct microbial inhibition for preserving meat and meat products since 1940 [52]. The radionuclides approved for food irradiation include 137Cs and 60Co, and radioactive cobalt (^{60}Co) decays to non-radioactive nickel by emitting high-energy particles and X-rays [36]. The X-rays kill rapidly growing microorganisms but do not leave the product radioactive because they are highly penetrating; therefore they can be used to treat food [66]. The benefits of using ionizing radiation in food preservation include high efficiency in inactivation of microorganisms, and that the ionized product is essentially chemically unaltered, and can be treated after packing in containers [52]. A maximum dosage of 10 kGy represents a low amount of energy which preserves the freshness and nutritional quality of the meat and meat products when compared with thermal methods [1].

Irradiated fresh meat color changes may be due to the inherent susceptibility of the myoglobin molecule to energy input and alterations in the chemical environment, heme iron being particularly susceptible. Brewer [66] stated that maintenance of a fresh meat color during the process of irradiation could be enriched by combinations of premortem supplementation of antioxidants to livestock, postmortem condition of the fresh meat prior to irradiation (pH, oxymyoglobin vs. metmyoglobin), addition of antioxidants directly to the meat, gas atmosphere, packaging, and temperature control. Radiation treatment resulted in essentially no loss of thiamine [67], therefore suggesting that such radiation has no detrimental effects on meat nutrients. However, capital costs and complicated technology are involved in irradiation.

9.4 PRE-RIGOR CHANGES

Rigor mortis is a temporary process occurring during the course of postmortem anerobic glycolysis and is characterized by progressive stiffening of the muscle and loss of ATP. When ATP is exhausted, the myosin and actin molecules remain locked together and yield the stiffness of muscle in rigor. The development of rigor mortis has been determined by several methods including loss of extensibility, muscle shortening, tension development, resistance to strain, and by a combination of muscle tension and shortening [31, 68]. The rate of postmortem glycolysis and the extent to which it occurs have significant implications in fresh meat quality. As anaerobic glycolysis proceeds from the point of slaughter to rigor mortis, various changes occur in the muscle. The production of H^+ leads to a more acidic condition, which, in turn, is measured as a decrease in meat pH. Fast-glycolyzing muscles yield higher tenderness scores compared with slow-glycolyzing muscle [69]. Pre-slaughter stress causes the depletion of muscle glycogen and therefore limits postmortem glycolysis, resulting in meat with an increased ultimate pH [70].

Interactions between pH and temperature during the onset of rigor directly influence meat quality through effects on proteolysis, protein denaturation, and myofibrillar shrinkage [71]. The calpain system is the most likely cause of myofibril-related tenderization, and proteolytic and/or autolytic activity of μ-calpain is largely a function of the interaction between pH and temperature [72]. Muscle temperature at pH 6.2 has been used as an important threshold in meat science because it could be an indirect indication of cold and heat shortening [73]. At slaughter, the muscle temperature is around ≅38–40°C; once the meat has been processed, it is placed into a cooler at 4°C. Temperature has a significant effect on muscle glycolytic reactions and rigor onset.

Pre-rigor meat has a higher water-holding capacity and better fat-emulsifying properties than post-rigor meat, which makes it more suitable for making processed meat products such as sausages. These properties can be maintained if pre-rigor meat is frozen quickly to temperatures below –20°C. However, when the frozen meat is thawed, it shortens and loses its water-holding capacity. Adding 1.8% salt to pre-rigor meat helps to maintain the pre-rigor attributes for several days when chilled [74]. With pre-rigor salting, the water-holding capacity is maintained due to a strong electrostatic repulsion between adjacent protein molecules caused by an initial combined effect of relatively high ATP concentration, high pH, and ionic strength. Adding salt pre-rigor inhibits ATP turnover but does not affect the rate of glycogen breakdown.

9.4.1 Cold Shortening

Cold shortening is a phenomenon that occurs in pre-rigor muscle and results in less tender meat. "Shortening" refers to the short sarcomere length characteristic of highly contracted muscle with protein denaturation and water loss [68, 75]. Rapid chilling may have a detrimental effect via cold shortening, which results in a drastic decrease in tenderness. The degree of overlap between myosin and actin filaments primarily contributes to meat toughening. Changes in angles of crisscross connective tissue lattice and crimp length are responsible in part for the relationship between sarcomere length and meat tenderness [76]. The toughness of cold shortened meat may also be due to the endogenous enzymatic tenderization mechanism and shortened sarcomere length. The effect of shortening sarcomeres on shear force is significantly detrimental when proteolysis is relatively slow. The "cold" refers to the rapid cooling which must occur in order to observe the effect (Figure 9.9).

If meat is frozen prior to rigor onset and subsequently thawed, it will shorten dramatically and be extremely tough. This phenomenon is referred to as "thaw shortening." The process of pre-rigor freezing can damage the sarcoplasmic reticulum and destroy its ability to regulate calcium concentrations within the myofiber. Both calpains and myosin-ATPase require free calcium ions in the cytoplasm for their activities [77]. It has been shown that calcium-reserving organelles lose their function at abnormal cellular temperature. During thawing, all the components necessary for muscle contraction are still present, but control of the reactions is lost. As a result, anaerobic metabolism processed at a very rapid rate and is concomitant with severe contraction.

9.4.2 Acceleration of Postmortem Glycolysis

Electrical stimulation is the post-slaughter application (pre-rigor) of an electrical current to the carcass to accelerate postmortem glycolysis of the fresh meat [78]. Electrical stimulation causes the muscle to undergo continuous contraction–relaxation cycles. The muscle's content of ATP, the compound needed to produce the energy required for muscle contraction, is being depleted, thus increasing the rate of glycolysis [72]. The variability in overall ATPase postmortem is primarily responsible for the variability in postmortem pH fall in muscle. This causes the muscle to replenish ATP by accelerating postmortem glycolysis. With the acceleration of postmortem glycolysis, a rapid build-up of lactic acid occurs,

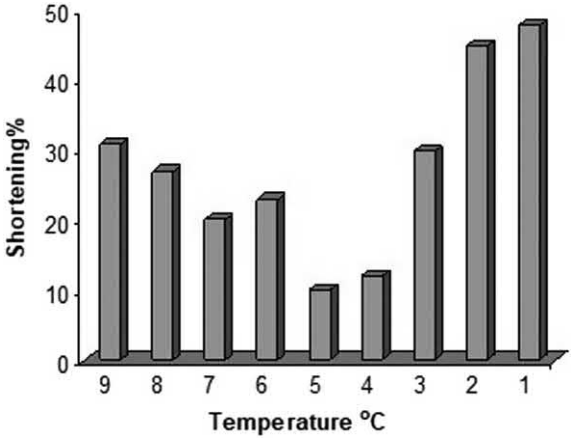

FIGURE 9.9 Changes in biochemical metabolites during the onset of rigor mortis. (From Newbold [160].)

and, in some cases, the pH of electrically stimulated muscle can reach a pH of 6.0 in a few hours instead of the 12 to 16 hr that may be required for non-stimulated muscles. The high energy of activation means that any cooling of the muscles will markedly increase the time for attainment of rigor mortis with a larger effect in stimulated muscle. Additionally, electrical stimulation causes muscular contraction sufficient to cause physical disruption of tissue. Acceleration of proteolysis could be classified as a secondary effect mediated through time/temperature–pH interaction, affecting factors such as enzyme stability and activity [72].

Electrical stimulation has been demonstrated to improve most aspects of meat quality including tenderness, color, and palatability. Chilling for 48 or 72 hr would maximize the quality of beef carcasses. However, most fresh meatpackers do not have the required facilities to hold the meat for this length of time before it is graded or shipped. Therefore, electrical stimulation plays an important role for the packer. There are three theories on the mechanism by which electrical stimulation tenderizes meat. First, because the onset of rigor mortis is hastened by electrical stimulation, muscle fibers do not shorten to the same extent as those from un-stimulated carcasses when exposed to cold-shortening temperatures. Second, because of the rapid drop in pH caused by the accelerated postmortem glycolysis while muscle temperatures are still high, conditions are favorable for the naturally occurring enzymes responsible for tenderization during the degradation of muscle proteins [79]. Third, histological images showed the appearance of contracture bands containing predominantly stretched, ill-defined, and disrupted sarcomere from electrically stimulated muscles [72]. Contractor bands, which may be caused by physical disturbance associated with stimulation-induced contractions, are also observed within some of the electrically stimulated muscle fibers. This structural damage may result in greater fragmentation of the muscle fibers upon chewing or mechanical shearing force, thereby increasing its tenderness [80, 81]. If the time interval between successive stimuli is more than approximately 0.25 s, the muscle titanic shortening is reversible [72]. On the other hand, when a higher frequency of current is applied, the muscle may not have enough time for relaxation between successive twitches, and this forms irreversible contracture bands. However, several researchers have disagreed with respect to the importance of structural damage in increasing the tenderness of electrically stimulated meat. In some studies, electron micrographs of electrically stimulated samples revealed protein precipitation, not structural damage. A number of studies have indirectly indicated that physical disruption had less effect on tenderness than did proteolysis [82]. It is clear that electrical stimulation favors autolysis of calpain rather than proteolytic activity [83]. There is also evidence suggesting that both physical disruption and effects on the calpain system arise as a consequence of stimulation.

There is evidence indicating that flavor is significantly improved by applying electrical stimulation [84]. This is attributed to the concentration of adenine nucleotides and their derivatives. Another advantage of using electrical stimulation is the reduction in aging time. Electrical stimulation resulted in a substantial decrease in the aging time needed to achieve a specified level of tenderness.

Meat retailers benefit from the use of electrically stimulated meat because of the improved appearance of retail cuts. Electrically stimulated fresh meat had a brighter color, less surface discoloration, and a more desirable overall appearance than non-stimulated meat [85]. Meat from electrically stimulated sides had a higher percentage of oxymyoglobin, the pigment responsible for the bright cherry-red color of beef, than meat from their non-stimulated counterparts. There is conflict on the persistence of color of electrically stimulated meat. The color-enhancing effect of high voltage electrical stimulation was reported not to persist beyond 24 h when subjectively scored. Persistent effects of high- and low-voltage electrical stimulation up to 6 days after postmortem treatment were also reported [85]. The improved appearance of the meat from electrically stimulated carcasses does not appear to be significantly related to its effects on bacteria [86].

9.5 POSTMORTEM CHANGES

Although the establishment of rigor mortis is generally accepted to be the point, the exact point at which the conversion of muscle to meat is completed is not easy to determine. The functional role of skeletal muscle is lost and rigor has been established, but the metabolic activity of the tissue would not stop. Many biochemical processes, some of which have significant implications for the meat quality characteristics, may still occur.

9.5.1 Meat Color

Color is the main trait critical to fresh meat purchase decisions [87]. Fresh meat color is governed primarily by myoglobin (Mb) content, its redox state, and interactions with several intrinsic and extrinsic factors [88]. The bright cherry-red color attractive to the consumer has been often associated with freshness of the meat, whereas a brownish discoloration is perceived as indicative of spoilage [89]. In general, the myoglobin concentration within a given fresh muscle will differ according to the species or age and is dependent on muscle fiber distribution [90, 91]. Oxidative muscles (more type I muscle fiber) exhibit greater Mb content [92] and mitochondrial concentration [93] than glycolytic (more type II muscle fiber) muscles. In postmortem oxidative fresh muscles, the mitochondrial metabolism surpasses myoglobin's ability to bind oxygen leading to accelerated metmyoglobin formation and surface discoloration [93]. Psoas major muscle contains a greater proportion of fiber type I (oxidative) than longissimus thoraces muscle [92], which is positively correlated to Mb content [92]. Furthermore, an increased level of Mb in muscles is associated with low color stability. The relative proportions of the three myoglobin forms, deoxymyoglobin (Mb), oxymyoglobin (MbO), and metmyoglobin (MetMb), depend on the oxygen availability and affect the color of fresh meat. The oxygen availability depends on the oxygen partial pressure, penetration, and consumption rate of the muscle

[94]. The penetration depth of light decreases as an effect of increased light scattering due to an increased amount of water outside the myofibrillar space induced by the pH drop during glycolysis [95]. The difference in pH dropping between muscles could be attributed to the difference in muscle fiber type proportion of the muscles [96]. Glycolytic muscles usually exhibit greater glycogen levels favoring increased postmortem accumulation of lactic acid than oxidative muscles [97]. The meat surface may be more or less translucent depending on the rate of postmortem pH drop, ultimate pH, and the extent of protein denaturation [95]. During postmortem glycolysis, the sarcoplasmic proteins denature and precipitate on the myofibrils, resulting in increased light scattering and less light penetration [95]. All these processes occur within the small heme portion of the larger myoglobin protein. When heme iron is in the ferrous form and lacks a sixth position, it is referred to as "deoxymyoglobin." The color of deoxymyoglobin is purplish-red, which is characteristic of fresh meat. Ferrous myoglobin that is exposed to air will bind oxygen at the sixth coordination site and from oxymyoglobin. Oxymyoglobin is cherry red and typical of fresh meat displayed in retail outlets. The process by which deoxymyoglobin binds oxygen and consequently is converted to oxymyoglobin is called "oxygenation." It is important that this should not be confused with "oxidation." The process of oxidation occurs in myoglobin when ferrous (+2) iron (deoxymyoglobin or oxymyoglobin) is converted to ferric (+3) iron and leads to the third form of myoglobin found in fresh meat, metmyoglobin. Metmyoglobin is brownish-red in color and is characterized by ferric iron with a water molecule bound at the sixth position. The oxidation of deoxymyoglobin or oxymyoglobin leads to the formation of metmyoglobin; this process occurs gradually over the surface of meat cuts during storage and/or display.

9.5.2 Lipid Oxidation

Lipid stability of pressure-treated foods of animal origin has been little investigated, and results are contradictory [36]. Rivas-Canedo et al. [98] used high pressure (400 MPa, 10 min at 12°C) to treat minced meat, which was packaged with or without aluminum foil in a multilayer polymeric bag. They found that pressurization produced significant changes in the levels of some volatile compounds presumably originating from microbial activity and the plastic material [98].

Although lipid in meat contributes significantly to flavor, its oxidation will result in the production of free radicals, which lead to the formation of rancid odors and off-flavor. Oxidation might also play a role in controlling the proteolytic activity of enzymes and could be linked to meat quality. The oxidative stability of meat depends upon the balance between anti- and pro-oxidants, including the concentration of polyunsaturated fatty acids [99]. It has been demonstrated that dietary fat and vitamin E supplementation can influence the antioxidant enzyme activities in meat [100]. Fatty acids are chains of carbon atoms with a carboxylic acid group at one end and vary in length according to the number of carbon atoms, which comprise their backbone and may be saturated or unsaturated.

TABLE 9.2
Fatty Acid Content of Muscle Foods

Species	% Saturated	% Monounsaturated	% Polyunsaturated
Beef	55.5	52.0	3.0
Pork	44.0	56.5	10.5
Mutton	55.0	41.5	4.0
Poultry	30.5	45	18.5
Fish	30.0	33.0	37.0
Goats	51.3	43.5	5.09

Source: Hultin [161].

Unsaturated fatty acids may contain one (mono-saturated) or several (polyunsaturated) double bonds between the carbon atoms and are generally liquid at room temperature. It should be noted that the proportions of saturated, monounsaturated, and polyunsaturated fatty acids in meat tissues depend on the species (Table 9.2). In monogastric species, such as pigs and chickens, they may be influenced by diet.

Saturated fatty acids are regarded as harmful to human health in contrast to polyunsaturated fatty acids, which play a favorable role in the prevention of some human artery diseases [99]. Therefore, increasing the proportion of polyunsaturated fatty acids in meat is currently recommended. Meat from monogastric animals contains high levels of unsaturated fatty acids relative to meat from ruminants. Meat fatty acid composition is influenced by a number of factors including muscle type and its oxidation [14]. The factors that make muscle lipids susceptible to oxidation are either intrinsic to the meat products or related to the technological process [101]. Oxidation of muscle lipids produces primary and secondary products such as hydroperoxides, free radicals, endoperoxides, malondialdehyde (MDA), epoxides, alkanes, hydrocarbons, alcohol, thiobarbituric acid reactive substance, and also acids that may be toxic to humans [102]. The oxidation of meat may be reflected in off-odors and flavors detected by sensory panels. It may also result in increased peroxide values or compounds, mainly MDA, giving a red color when reacted with thiobarbituric acid. Increasing aging time from 8 to 15 days increased the levels of MDA due to normal oxidation processes occurring in refrigerated meat. Differences in MDA between meat samples aged for 8 and 15 days were still detected after 4–8 months of frozen storage. Initial storage conditions may affect the subsequent lipid stability of frozen meat regardless of the storage temperature [103]. At –20°C storage temperature, the MDA content increased with increased storage time [104]. The low level of hydroperoxides in fresh meat increases rapidly to reach a maximum after several months of freezing. The threshold value for rancidity is 1–2 mg of MDA per kg of meat [105]. However, consumers are unlikely to detect off-flavor at values below a threshold of about 0.5 mg MDA/kg [106].

The double bonds located within polyunsaturated fatty acids are sites of chemical activity. Oxygen is a key element

for lipid oxidation and may react with these sites to form peroxides, which lead to rancidity. Polyunsaturated fatty acids are susceptible to rancidity as they contain double bonds. Meat with high concentrations of polyunsaturated fatty acids can develop a rancid flavor faster than meat with less polyunsaturated fatty acids. The interaction of oxygen with polyunsaturated fatty acids is a non-enzymatic process. Vacuum packaging of meat products therefore provides a longer shelf life by excluding oxygen from the packaging.

Enzymatic-based lipid oxidation occurs in meat and is also known as microsomal lipid oxidation. This process requires certain biochemical cofactors including reduced forms of nicotine adenine dinucleotide phosphate or nicotine adenine dinucleotide, adenosine diphosphate, and iron ions. The enzymatic nature of the process implies the involvement of membrane-bound proteins. The cooking of meat provides sufficient heat to denature enzymes, and therefore, enzymic/microsomal lipid oxidation will not occur in cooked meats. During normal physiological functioning, the enzymes found in these sub-cellular organelle membranes produce chemically reactive substances known as radicals. These are a necessary part of normal cell functioning, and in the "living state" the cell has a variety of mechanisms for protecting itself against the undesirable actions of radicals. In postmortem, many of these protections are lost; therefore radicals may hasten lipid oxidation and consequently cause rancidity.

Color deterioration and lipid oxidation may be linked, although the precise mechanisms are still unclear [107]. Some control over increased susceptibility to oxidation can be attained by feeding with higher levels of vitamin E, as an antioxidant active in meat [108]. The delaying of myoglobin oxidation is accomplished in a variety of ways. These include storage and display of meat under refrigerated conditions, hygienic preparation of meat cuts, and selective use of lighting. In addition, the application of antioxidants, such as ascorbic acid (vitamin C), citric acid, or γ-tocopherol (vitamin E), may extend the color shelf life.

9.6 MEAT STORAGE AND SAFETY

9.6.1 Refrigeration

The development of refrigeration had more impact on meat preservation than any other technological advancement. Fresh meat refrigeration storage above or below the freezing temperature has been the traditional preservation method. Ultra-chilling technology, which keeps fresh meat just above the freezing temperature, has been used with success [109]. Chilling is critical for fresh meat hygiene, safety, shelf life, and quality characteristics. Chilling air reduces the temperature of fresh meat surfaces and enhances meat drying, which reduces the growth of bacteria [110]. However, it is difficult to remove heat quickly from the deeper tissue of the fresh meat. Proper refrigeration, therefore, not only lowers the temperature of the harvested fresh meat but also slows down the rate of glycolysis as the temperature is decreased. Modern ultra-chilling coolers operate at lower relative humidity than in the past to reduce condensation, which is a source of contamination. The term ultra-chilling is used to describe a process where a minor part of the product's water content is frozen [111]. The lower relative humidity enhances the evaporation of water, which is required for the absorption of heat from the meat surface, speeding up the postmortem fresh meat chilling process and minimizing microbial growth. The use of a blast chilling system and rapid air movement at 1–5 $m.s^{-1}$ for 1–5 h will rapidly cool fresh meat, reducing cooler operation time. Moreover, rapid meat chilling increases meat product yield due to lower surface evaporation, while the rapid drying of the meat surface helps to reduce bacterial growth. Controlling airflow inside industrial meat chillers is of paramount importance because it determines both the efficiency and the homogeneity of meat chilling [112]. Therefore, the use of ultra-rapid chilling results in lower shrink loss that accompanies a reduced chilling period [113]. During ultra-chilling, the temperature of the product is lowered, often 1–2°C, below the initial freezing point of the meat product. The refrigeration technology will maintain the meat temperature between 0°C and the temperature at which ice crystals form in the product (–2°C), and will further extend the shelf life of chilled fresh meats [114]. After initial surface freezing, the ice distribution equilibrates and the product obtains a uniform temperature at which it is maintained during storage and distribution [111]. At ultra-chilling temperatures, most microbial activity is inhibited or terminated and the chemical and physical changes may progress or accelerate. The ice crystals in ultra-chilled meat products protect the fresh meat from temperature rises in poor cold chains; however, some increase in product drip loss may occur during storage [111]. The process involves water chilling of fresh meat and air freezing at –15°C for approximately 30 min and then the packaging of the products. They are again placed in an air freezer to achieve the required meat temperature and then stored and distributed at –1 to –2°C [111]. Ice crystals forming and recrystallization can cause microstructural changes to meat tissue during freezing, resulting in cell dehydration, drip loss, and tissue shrinkage during thawing. Cathepsins B and B+L remained active at the selected storage temperatures, which may lead to softening during subsequent chilled storage. Ultra-chilling of fresh meat at –2.0°C improved the shelf life significantly compared with traditional chilling at +3.5°C [115]. The ultra-chilled fresh meat maintained good sensory quality and low microbiological counts during the whole storage period (16 weeks), while the shelf life of chilled samples was just 14 days.

Ultra-rapid chilling of pre-rigor meat may lead to cold-shortening and toughening [36]. Therefore, James et al. (1992) [116] stated that the ultra-chilling of fresh meat may produce a darker lean color than control meat. The application of blast chilling to fresh meat may result in cold-induced toughening and therefore compromise meat quality. This is due to subtle changes in the rate and extent of pH decline during chilling. An impairment of autolytic enzyme system functions may be responsible for this toughening in addition to the expected effects of cold shortening. At a constant rigor temperature of 35°C, almost 80% of the μ-calpain activity was lost during

rigor development, while only about 20% of the activity was lost when meat was exposed to a constant rigor temperature of 15°C [117]. This inactivation process could be the explanation for the differences in quality between meats with fast and slow pH time courses when exposed to the same chilling regime [118]. However, ultra-rapid chilling of carcasses (–20°C) was reported to produce meat as tender as that from carcasses chilled at 4°C and reduced evaporative weight losses by 0.5–1% [119]. Therefore, blast chilling may be best used in conjunction with electrical stimulation to accelerate the onset of rigor mortis in order to avoid the development of cold shortening.

9.6.2 Aging and Meat Quality

Historically, fresh meat has been aged to improve its quality characteristics. Aging is necessary as meat is often unacceptably tough immediately following rigor onset. The time required for aging varies with the type of muscles. Recently, Colle et al. [120] aged beef strip loin and top sirloin for up to 63 days and concluded that in order to optimize the consumer's perception of tenderness the strip loin does not need to be aged past 14 days, while the top sirloin should be aged for at least 21 days. The ideal storage temperature may be applied in the pre- or post-rigor state and is very effective in improving meat tenderness. The aging processes originate within the myofiber and are responsible for the degradation of cellular constituents.

The analysis of muscle proteins along with meat quality traits during chilled aging is crucial in understanding the biological basis of changes in meat quality. The proteolytic enzymes in meat that have been most studied are the cathepsins and calpains. Aging between 6 and 43°C had significant effects on hunter L*, shear force values, and drip loss [121]. The most relevant consequence of aging is an improvement in meat tenderness [76]. Meat tenderness increased with longer aging for certain muscles, while no difference was observed for other muscles. Fresh biceps femoris muscles and semimembranosus muscles should be aged for at least 14 to 21 days, respectively, to optimize consumer perception of tenderness [122]. They concluded that the tenderness of biceps femoris muscle increased from 53% after 2 days of aging to 63% after 14 days of aging to 75% after 21 days of aging. The improvement in the tenderness of biceps femoris may be due to the breakdown of myofibrillar proteins. The tenderization process involves complex changes in muscle metabolism in the post-slaughter period and is dependent on animal breed, metabolic status, and environmental factors, such as the rearing system and pre-slaughter stress. During aging, the structures of the myofibrillar and other associated proteins undergo some modifications, and collagen is weakened to a lesser extent [122]. The degradation of nine actin and/or actin-relevant peptides out of 20 identified ones is related to meat quality traits during aging [121]. The proteolytic enzymes in meat play a significant role in improving meat tenderness during aging. Enzymes require specific conditions such as temperature and pH for optimal activity, and these can be determined and maximized in meat to improve tenderness. Cathepsins are effective proteolytic agents that have been identified in meat and located within lysosomes and operate best at pH <5.2 values. Myofibrillar proteins are degraded when incubated with various cathepsins *in vitro*. It is believed that catheptic enzymes are able to act on the pH of meat to produce tender meat by degrading myofibrillar proteins.

Calpains are proteases that require calcium ions (Ca2+) for activity. There are two types of calpains found in sarcoplasm: one requires a high concentration of free calcium for activation, and another one requires a low concentration of free calcium. The amount of calcium available in normal muscle cells excludes the high-calcium-requiring calpain as a major contributor to meat tenderization. Both types of calpains require a high pH, <6.6, for optimal activity. This value is substantially higher than the pH 5.6 of normal meat; therefore the maximum activity of calpains would most likely occur during the early postmortem condition.

The degradation of cytoskeletal proteins such as desmin, vinculin, titin, and nebulin was considered to be responsible for changes in the water-holding capacity during aging [123]. The formation of drip is generally considered to be a result of the denaturation of contractile proteins and shrinkage of myofibrils during rigor development [122]. On the other hand, reduced drip loss was related to the "leak-out" effect, and aging itself did not improve water-holding capacity. Higher rigor temperature accelerated drip loss during vacuum-packed storage, and drip loss increased at a high pH (6.2) as aging time lengthened [121]. The effect of early postmortem pH and temperature on meat quality is dependent on aging time. In addition, this affects meat color by influencing the surface reflectance [124]. Injection with calcium/sodium chloride after slaughter accelerated postmortem tenderization and increased the tenderness of the meat sample apparently by enhancing the activity of the endogenous calcium-dependent proteases (m- and μ-calpain) [125].

Flavor intensity increased with aging time [126]. This may be due to postmortem processes such as proteolysis and lipolysis resulting in the development of flavor precursors. Many peptides are produced during aging [104]. They can react with other molecules thus producing new flavor compounds. Another source of volatiles in meat with age is the degradation of lipid. Lipid oxidation can adversely affect fresh meat product flavor [122]. During cooking, these compounds may be oxidized further and react with Maillard products to give many other compounds that may contribute to flavor [127]. Therefore, aging meat for the development of flavor, aside from its tenderizing effect, becomes a questionable practice for meat that is to be held in zero storage for more than six months. The holding period has a direct effect on the storage life because it permits oxygen absorption by the exposed fat.

9.6.3 Packaging

Packaging protects products against deteriorative effects, which may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity,

and other measurable factors [36]. The factors influencing the shelf life of packaged fresh meat are product type, gas mixture, package, and headspace, packaging equipment, storage temperature, and additives. Modern fresh meat packaging is minimally permeable to air moisture, and so surface desiccation is prevented, while gas permeability varies with the particular film type used. Packaging options for fresh chilled meat are air-permeable packaging, low-O_2 vacuum, low-O_2 MAP with anoxic gases, and high-O_2 MAP. While air-permeable packaging is not MAP, the use of overwrapped packaging materials within the master pack or tray-in-sleeve systems allows for this packaging option to be a component of MAP [128].

Vacuum packaging materials for fresh meat cuts are usually three-layered co-extrusions of ethyl vinyl acetate/polyvinylidene chloride/ethyl vinyl acetate. Vacuum packaging has an O_2 permeability of less than 15.5 ml m^{-2} (24 h) at one atmosphere as a result of the polyvinylidene chloride layer. The absence of O_2 in fresh meat packages may eliminate aerobic microbial growth, which usually causes a brownish color due to the deoxymyoglobin state of myoglobin. Low-O_2 vacuum packages for fresh meat cuts involve placing the retail cut in a barrier polypropylene tray and vacuum sealing barrier films that are heat shrunk to conform to the shape of the product [129]. Vacuum packaging removes the air from the package with gaseous mixtures such as N_2, CO_2, or mixtures of N_2 and CO_2 before heat sealing the film layers [130]. The lidding film of vacuum packaging contains outer and inner air-permeable layers; the outer film layer is usually peeled away from the permeable layer so that O_2 can then contact the meat surface and result in a bloomed color [129, 130]. Mechanisms of intelligent packaging are well-discussed [131].

Modified packaging for display fresh meat requires a barrier of moisture and gas permeation through filming materials to maintain a desirable condition during storage. Although meat vacuum packaging is the most effective packaging, the use of low-O_2 modified packaging is limited [132]. Low-O_2 modified packaging may be used as a barrier package with an anoxic atmosphere of N_2 and CO_2. Nitrogen is not reactive with meat myoglobin; therefore, it maintains the integrity of the fresh meat. The meat pigments become oxygenated when the overwrapped permeable film package is removed from the master pack for retail display [129]. If the air-permeable film does not permit sufficient O_2 for oxymyoglobin formation, microperforated shrink films with additional holes can be used to promote faster meat blooming [133]. Carbon monoxide (CO) has also been used in low-O_2 meat vacuum packaging systems. Small amounts of CO in the packaging system can be sufficient to impart a desired red meat color [129, 134, 135]. The high O_2 (80%) in fresh meat modified packaging is allowed sufficient shelf life for processors and retailers with controlled distribution systems [134].

Active packaging for fresh meat involves the interaction of specific compounds with the contents and atmosphere to maintain or extend meat quality and shelf life [136]. Active meat packaging may also involve the deliberate altering of the atmosphere at a specified time through passive or active monitoring needed with controlled atmosphere packaging [137]. On the other hand, intelligent meat-packaging systems contain components that sense the environment and process the information and then allow action to protect the meat by conducting communication functions. Active and intelligent packaging functions include moisture control, O_2-permeable films, O_2 scavengers, O_2 generators, CO_2 controllers, odor controllers, flavor enhancement, ethylene removal, antimicrobial agents, microwave susceptors, indicators of specific compounds, and temperature control packaging [138, 139].

Antimicrobial packaging is the mixture of antimicrobial substances in meat-packaging materials to control the undesirable growth of microorganisms. The antimicrobial packaging system is an advanced technology to extend shelf life and improve meat safety in both synthetic polymers and edible films [132]. According to [140], antimicrobial packaging films can mix the antimicrobial substances into a sachet connected to the meat package from which the bioactive substance is released during storage, or the antimicrobial can be spread into the packaging film (Table 9.3). The sachets can include O_2 scavengers, CO_2 generators, and chlorine dioxide generators, while bioactive agents dispersed in the packaging may be O_2-scavenging films, silver ions, triclosan, bacteriocins, spices, essential oils, enzymes, and other additives [141]. The active agents can be released onto the meat surface and they will migrate slowly into polymers used for meat packaging. Volatile active substances in the packaging can move into the gaps between the package and the surface of the meat [142].

Potential antimicrobial agents for use in meat-packaging systems are organic acids, acid salts, acid anhydrides, parabenzoic acids, alcohol, bacteriocins, fatty acids, fatty acid esters, chelating agents, enzymes, metals, antioxidants, antibiotics, fungicides, sterilizing gases, sanitizing agents, polysaccharides, phenolics, plant volatiles, plant and spice extracts, and probiotics [143]. Antimicrobial compounds that have been evaluated in film structures are organic acids and their salts, enzymes, bacteriocins, triclosan, silver zeolites,

TABLE 9.3
Natural Active Components Combined Directly into Polymers Used for Meat Packaging

Active Component	Polymer	Substrate	References
Nisin	Polyethylene	Beef meat tissue	[162, 163]
Lactic acid	Alginate	Beef meat tissue	[164]
Vit. E	Low-density polyethylene	Beef meat	[165]
Rosemary extract	Polystyrene	Lamb meat	[166]
Thyme, rosemary, and sag spice	Caseinate and whey protein	Beef minced meat	[167]
Oregano extract	Polystyrene	Lamb meat	[166]
Chitosan	Chitosan	Culture media	[168]
Triclosan	Plastic matrix	Meat surface bacteria	[143]

and fungicides [142]. However, triclosan (500–1000 mg kg^{-1}) in polyethylene films exhibited antimicrobial activity against pathogenic bacteria in agar diffusion assay but did not effectively reduce microorganism growth on meat in vacuum packaging at 7°C [144]. The examination of four polyethylene films coated with three different bacteriocins showed antimicrobial activity against most of the microorganisms [145]. Antimicrobial agents such as nisin and chlorine dioxide have shown effectiveness against bacteria [140]. Fast- and slow-release ClO_2 sachets reduced total plate counts by 1–1.5 logs in packages of meat after 15 days, with no off-odor detected by sensory panelists, but the color of chicken adjacent to the ClO_2 was adversely affected [146]. The combination of rosemary extract and polypropylene film enhanced the stability of myoglobin and meat by the inhibition of metmyoglobin and lipid oxidation [147].

Bioactive edible coatings incorporate an antimicrobial compound in an edible coating, applied by dipping or spraying onto the meat products. Edible coatings of polysaccharides, proteins, and lipids can improve the quality of fresh, frozen, and processed meat products by delaying moisture loss, reducing lipid oxidation and discoloration, enhancing product appearance, and functioning as carriers of food additives [148].

9.6.4 Freezing

The large-scale preservation of meat by freezing commenced about 1880 when there was a surplus of meat and freezing offered a means of preserving meat for a certain period [149]. The advantages of temperatures below the freezing point were in prolonging the useful storage life of meat and in discouraging microbial and chemical changes [52].

Fast freezing produces minute intracellular ice crystals and thus diminishes drip on thawing. The rate of freezing is dependent not only on the bulk of the meat and its thermal properties, but also on the temperature of the refrigerating environment, on the method of applying the refrigeration, and, with smaller cuts of meat, on the nature of the wrapping material used. A temperature of –55°C is ideal for the storage of frozen meat to completely prevent quality changes [150]. The swelling and shrinking of myofibrils are well-studied in the mechanism of the water-holding capacity in meat [151]. Low freezing temperatures will minimize enzymic reactions, oxidative rancidity, and ice recrystallization. Cryogenic freezing technology will significantly reduce freezing times compared with the conventional technique due to the differences in temperatures between the two techniques and the high rate of surface heat transfer resulting from the boiling of the cryogen. The cryogenic freezing technique doesn't need mechanical refrigeration, but a cryogen tank and suitable spray equipment will be enough. However, there may be some distortion of the shape of the meat products caused by the cryogenic process that might impact on the commercial application. Moreover, the cost of the cryogenic liquid is relatively high; therefore, it may limit its commercial use [152]. Steak palatability is slightly affected by thawing methods [153].

Freezing is a common practice in preserving meat quality for an extended time. Although freezing meat will minimize deterioration in meat color, flavor, and texture, the disadvantages of frozen meat are freezer burn, dehydration, rancidity, drip loss, and product bleaching [36]. Many meat products go directly from the freezer to cooking, in which case they are difficult to distinguish from fresh cuts [154] and the consumer is not able to differentiate. The shelf-life extension and the purchasing and inventory flexibility offered by frozen meat items are valuable assets in the meat service industry. Drip loss is one of the most important problems in frozen meat [51]. The loss of fluid generally reduces the eating quality, binding ability, and the weight of meat. The amount of drip loss (85% of the water in muscle) on thawing frozen meat has been related to the size and location of ice crystals in frozen meat, which are generally considered responsible for the changes in meat quality. When the fresh meat is frozen, muscle water associated with muscle protein is replaced with protein [155], which leads to decreased water-holding capacity after thawing. The most critical temperature in thawing meat is between –10 and –2°C; therefore, meat must rapidly pass this range [156]. The rapid chilling of fresh meat compromises the ability of sarcoplasmic reticulum and mitochondria to retain calcium [36].

Although freezing acts as a preservation technique to eliminate the meat enzyme activities and inhibit the growth of spoilage microorganisms, it initiates several physical and chemical changes in meat that lead to the deterioration in quality [157]. The deterioration of frozen meat quality is associated with ice crystal formation. It has been mentioned that at very low temperatures, recrystallization is very slow and equilibrium is approached while the crystals are small, while at temperatures near the melting point, recrystallization is rapid [36]. The rate of crystallization and the size of the crystals formed depend upon the temperature [158]. Slow freezing causes the water to separate into pools that form large crystals, which may cause greater structural damage surrounding tissue associated with larger intercellular ice crystals [159]. However, rapid meat freezing causes little water separation and produces small crystals. At very low-temperature freezing, there is practically no pool crystallization; the drip loss is less than from meats frozen at higher temperatures. Variations in temperature during storage cause recrystallization which may explain the deterioration in meat quality characteristics over frozen storage [77]. Recrystallization involves changes in the number, size, shape, orientation, or perfection of crystals following the completion of initial solidification [36]. The lower the temperature, the greater the inhibitory action and the longer the period of satisfactory storage. Most of the vitamin loss is caused by heat or light or is lost in the juices that escape. Muscle protein denaturation at low temperatures is similar to the denaturation of proteins at higher temperatures, which leads to loss of water. The solubility of myofibrillar proteins is lower in slowly frozen meat compared to fast-frozen meat [159]. Drip loss from thawing meat includes proteins, vitamins, and other nutrients, in addition to moisture, and results in decreased cooked yields and juiciness.

9.7 CONCLUSION

Much has been learned in recent years about the pre- and post-rigor conditions and their role in the determination of meat quality characteristics. This chapter aimed to describe the muscle structure, pre- and post-rigor mortis, and technologies for the preservation of fresh meat. In addition, it also presents important opportunities for ultra-chilling methods to reduce the use of freezing/thawing for the preservation of fresh meat and its capability for prolonging shelf life and improving meat quality and safety. A non-thermal technology can inactivate meat spoilage microorganisms and their enzymes at low temperatures without changing the quality characteristics of the meat and meat products. Further, meat-packaging technology is also reviewed in this chapter, as it incorporates compounds into packing systems to maintain or extend meat quality and shelf life. Natural compounds can replace chemical preservatives to provide the opportunity for "green labeling" to attract more consumers. New technologies are applied to extend the storage life of fresh chilled meat by the control of the hygienic conditions and temperatures of the product to supply high-quality, safe, and wholesome meat and meat products to consumers.

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10 Broiler Meat Production and Postharvest Quality Parameters

Isam T. Kadim, Issa S. Al-Amri, Abdulaziz Y. Al-Kindi, Msafiri Mbaga, and Amera K. Nasser

CONTENTS

10.1 INTRODUCTION

Broiler meat production represents one of the largest food industries worldwide. It has experienced larger changes over the past 100 years than over the past two millennia. These have led to an increase in broiler meat consumption and made it very popular all over the world. It is difficult to predict how the industry will develop in the future, but, no doubt, attention will be aimed at improving meat quality, quantity, and the processing of products. Moreover, due to world population growth and increasing income levels, the production of broiler meat has increased over the past few decades. Broiler chickens are reared intensively in most areas of the world to provide a high-quality protein to meet the global animal protein demand. Intensive rearing of broilers is one way of rapidly increasing animal protein supplies for rapidly increasing populations.

Commercially, broilers are housed in environmental-controlled confinement to create optimal conditions in order to manipulate day-length to maximize production. Broiler chicken meat is gaining popularity because of its competitive price, short production period, high rate of productivity, and positive nutritional image. Therefore, worldwide poultry meat production and consumption have increased rapidly, and, in most parts of the world, per capita consumption of poultry meat will continue to grow [1]. Broiler meat has maintained its identity and high value compared to other meats for personal health reasons. It is expected that by 2020 broiler meat production will surpass the production of all other meats [2]. The competitive prices of broiler meat compared to other meats, the absence of cultural or religious taboos, and dietary and nutritional properties are the main factors for poultry meat's attractiveness [3]. Broiler meat also fits the current consumer demand for low-fat meat with high unsaturated fatty acids and low sodium and cholesterol levels. According to Barroeta [4] broiler chicken meat may also be considered a "functional food" via the presence of bioactive substances with favorable effects on human health. These include conjugated linoleic

acid, vitamins and antioxidants, and a balanced omega 6 to omega-3 PUFA ratio.

Changes in the consumer's lifestyle have led to the manufacturing of processed meat products. This has resulted in significant investments in the broiler meat processing industry, increasing the availability of a large variety of processed ready meals [1]. The broiler meat processing industry started to use automatic machines to an even greater degree mainly due to the increasing consistency of raw meat cuts. Nuggets and patties production usually starts with a machine that produces a few hundred identical nuggets/patties every minute (Figure 10.1). Today more automation is seen in the cutting and slicing operations. This chapter highlights the potential development postharvest of the broiler chicken to provide high quality and healthy meat and meat products.

10.2 BROILER CHICKEN PRODUCTION

10.2.1 Populations

Chicken meat is one of the most popular and leading meat products in the world today. This is evidenced by the number of chickens produced globally each year. According to FAO_{STAT} [5], more than 20 billion broiler chickens were raised in 2013. Between 2000 and 2013 for example, a total of approximately 251.3 billion chickens were raised in the world. In economic terms, the production of these billions of chickens has both backward linkages (industries supplying raw materials and inputs to chicken production facilities) and forward linkages (processors, grocery stores, and final consumers). This process generates significant economic activities, tax revenues, and job creation.

Region-wise, Asia leads in terms of chicken production, followed by the Americas, Europe, Africa, and Oceania. In 2013, for example, these regions produced 11.6, 5.3, 2.1, 1.8, and 0.13 billion chickens, respectively. Between 2000 and 2013 a total of 20.7, 68.1, 134.9, 26.1, and 1.6 billion chickens were raised in Africa, the Americas, Asia, Europe, and Oceania respectively. This translates to 8.2, 27.1, 53.6, 10.4, and 0.7% for the five regions, respectively (Figure 10.2).

China was the top chicken producer in the world in 2013; it produced 4.84 billion chickens, followed by the United States (1.92 billion), Indonesia (1.79 billion), Brazil (1.25 billion), and India (0.71 billion). Overall, between 2000 and 2013 these top five chicken producers produced 131.6 billion chickens out of the 251.3 billion chickens produced in the world during that period, which accounted for 52% of global chicken production during that period.

10.2.2 Meat Production

Broiler chicken meat is reported to be a budget-friendly form of protein. Health-wise, chicken meat is naturally low in sodium, and without the skin it is naturally low in fat, providing only 70 milligrams of sodium per 85 g portion. According to the Organization for Economic Co-operation and Development [6], broiler meat is becoming the world's most consumed meat due to its low cost and its being the most accessible type of meat in the world. It is also free of many cultural barriers that affect consumption of other meats such as pork and beef. That is why some of the world's largest chicken-eating countries per capita are those which consume no pork at all. These include Israel and most Muslim countries. Furthermore, chicken production is much more friendly to the environment than most of the other kinds of meat. According to a recent study by the Environmental Working Group, chicken's carbon footprint per kilogram consumed is approximately half that of pork, quarter that of beef, and nearly one-seventh that

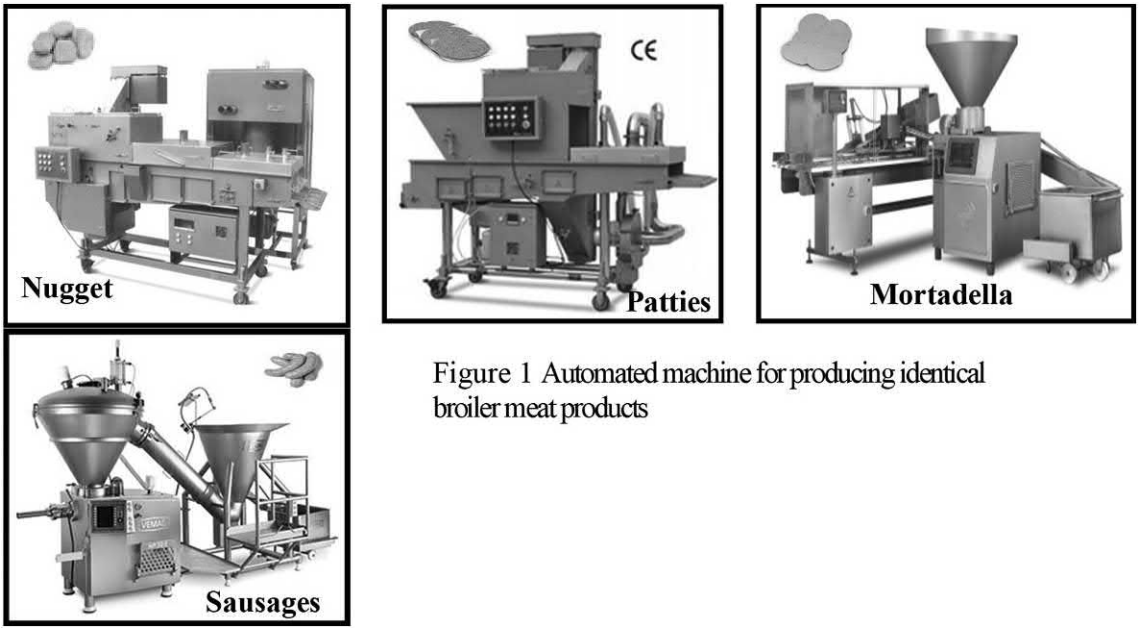

Figure 1 Automated machine for producing identical broiler meat products

FIGURE 10.1 Automated machine for producing identical broiler meat products.

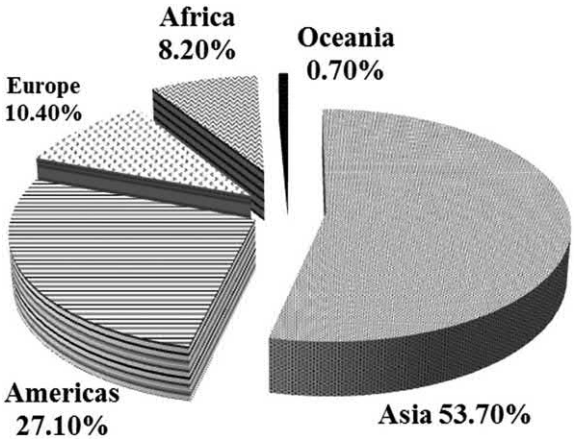

FIGURE 10.2 Regional share of chicken production total for the period from 2000 to 2013. (From FAOSTAT [5].)

of lamb. These qualities and attributes of chicken meat are a reason for the global popularity of chicken in the eyes of consumers, and its increased demand, which has translated to increased chicken meat production globally.

In 2013 approximately 96.3 million tons of indigenous chicken meat and 84.6 million tons of broiler chicken meat were produced globally. In addition, forecasts by USDA and other stakeholders put the expected 2015 global indigenous and broiler chicken meat production at 99 and 87.3 million tons respectively. Between 2000 and 2013, a total of approximately 1,067,109,627 tons of indigenous chicken meat were produced in the world.

Region-wise, the Americas are leading in terms of chicken meat production, followed by Asia, Europe, Africa, and Oceania. In 2013, these regions produced 42.1, 32.21, 16.1, 4.7, and 1.26 million tons, respectively. Furthermore, between 2000 and 2013 a total of 51.68, 483.96, 348.67, 169.14, and 13.64 million tons were produced in Africa, the Americas, Asia, Europe, and Oceania respectively. This translates to 4.8, 45.4, 32.7, 15.9, and 1.3%, respectively, for the five regions.

With respect to the top five chicken meat producers in the world, available statistics indicate that the United States tops the list whereby in 2013 the country produced 17.55 million tons, followed by China (13.35 million tons), Brazil (12.43 million tons), India (2.34 million tons), and Indonesia (1.84 million tons). Overall, between 2000 and 2013 these top five chicken producers produced 543.2 million tons out of the 1.067 billion tons produced in the world during that period. In other words, the top five chicken producers accounted for 51% of global meat production during that period. Trends in broiler chicken meat production indicated a steady increase between 2011 and 2014, while forecasts for the period between 2015 and 2020 also predict a steady increase.

10.2.3 Meat Consumption

Total global broiler meat production has increased by 400% over the past five decades. It is expected that within the next 10 years it will increase by another 25% due to the world's population increasing by 1 billion people, especially in developing countries. Although, poultry meat is generally popular around the world, there are major differences in consumption trends between countries such as Brazil and the United States (which consume 44.4 and 41.0 kg meat/capita/year, respectively) and countries such as China and India (which consume 11.1 and 2.0 kg meat/capita/year, respectively). Differences in broiler meat consumption may be due to differences in income, availability of the products, tradition, and eating habits [7]. The increase in ready-to-cook broiler meat is illustrated in Figures 10.3 and 10.4.

10.2.4 Meat Improvement

The poultry industry has seen major improvements in the genetics, health, husbandry, and processing segments. In 1925 it took, on average, 112 days to grow a broiler chicken to 1.14 kg live body weight. In 2010, it took only 47 days to grow to 2.60 kg market weight and in 2014 it reached 2.8 kg. It should be noted that in the 1920s chickens were grown in small farms and used for egg and meat production. However, when the poultry industry started to grow and specialize, egg and meat production breeds emerged and farmers began to specialize in one or the other. Genetic selection for efficient meat-producing breeds has resulted in an improved feed conversion ratio (kg feed/kg meat) from 4.70 to 1.92. In addition, developments in veterinary medicine and hygiene have helped to reduce mortality rates from 18 to 4%. These improvements over the years along with innovation/modernization in the primary processing sector [8] and in agriculture in general (more corn/soy/acre) have resulted in consumers paying less today for poultry meat than they did 25 years ago. However, it should be noted that the proportion of breast meat in the carcass has also increased.

10.2.5 Changes in Meat Consumption Patterns

In general, there has been a 15% increase in meat consumption. However, the consumption of broiler meat has dramatically increased from 23% in 1965 to 50% in 2015 while the consumption of red meat has decreased from 72% to 50% during the same period. Broiler processed products have increased considerably since 1960, when a limited selection of broiler products such as hot dogs, frankfurters, and luncheon was available on the market [9]. The introduction of chicken nuggets in the 1970s was a tremendous advancement in the poultry industry. Subsequently, the introduction of pre-portioned broiler meat products has helped to increase consumption, in particular of more convenient packages with specific cuts/portions. In the 1960s, 85% of the market consisted of whole broiler chickens, whereas in 2015 they represented less than 10% because consumers today are willing to pay for the convenience of smaller portions with bone and skin already removed. Due to the improvement in automation and mechanization, line speed in broiler plants has increased from 3,000 birds per hour in 1970 to 13,500 in 2015.

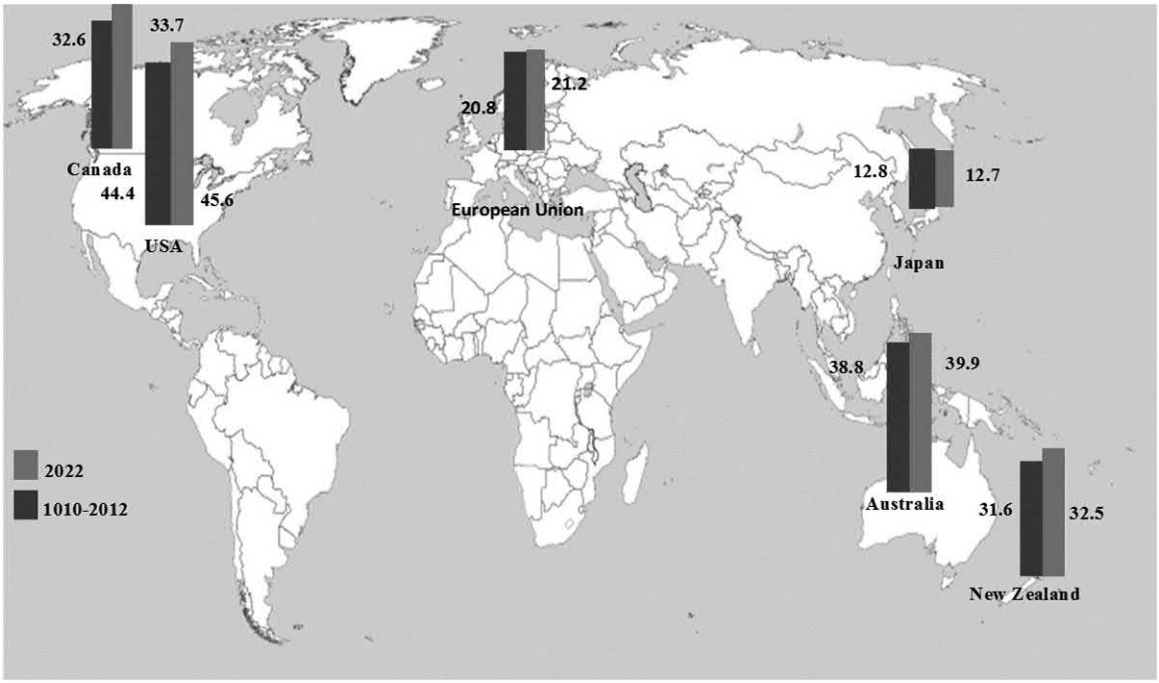

FIGURE 10.3 Demand in the United States, European Union, Japan, Australia, and New Zealand is satiated broiler meat consumption per capita, kg average 2010–2012 (estimate) and 2012 (forecast). (From OECD-FAO [6].)

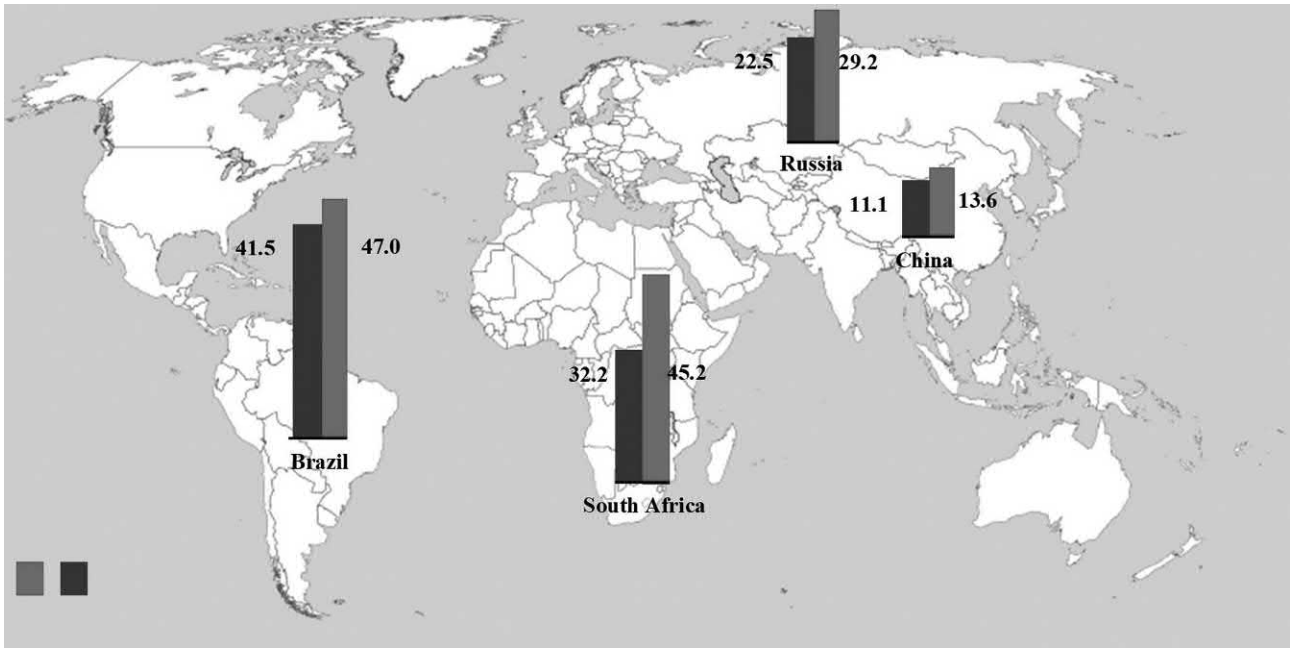

FIGURE 10.4 Demand in Russia, Brazil, China, and South Africa is satiated broiler meat consumption per capita, kg average 2010–2012 (estimate) and 2012 (forecast). (From OECD-FAO [6].)

10.3 BROILERS' SKELETAL MUSCLES

The skeletal muscles of a broiler chicken are shown in Figure 10.5. They range in size from small muscles to large muscles. White and dark meat in broilers represents breast and leg meat, respectively. These muscles comprise 40 to 50% of the average body mass of a broiler chicken. The major muscles in broilers are the leg muscle (biceps femoris) and breast muscle (pectoralis major). Skeletal muscles are also known as striated muscles because of their striated appearance when viewed under a light microscope. Figure 10.5 also shows a schematic diagram of whole muscle that is broken down into its components. A large muscle is composed of numerous muscle bundles covered by epimysium. Each muscle bundle is separated from the others by a connective tissue layer called perimysium. The functions of blood vessels and nerves supply energy to the active muscle and control its movement. The muscle

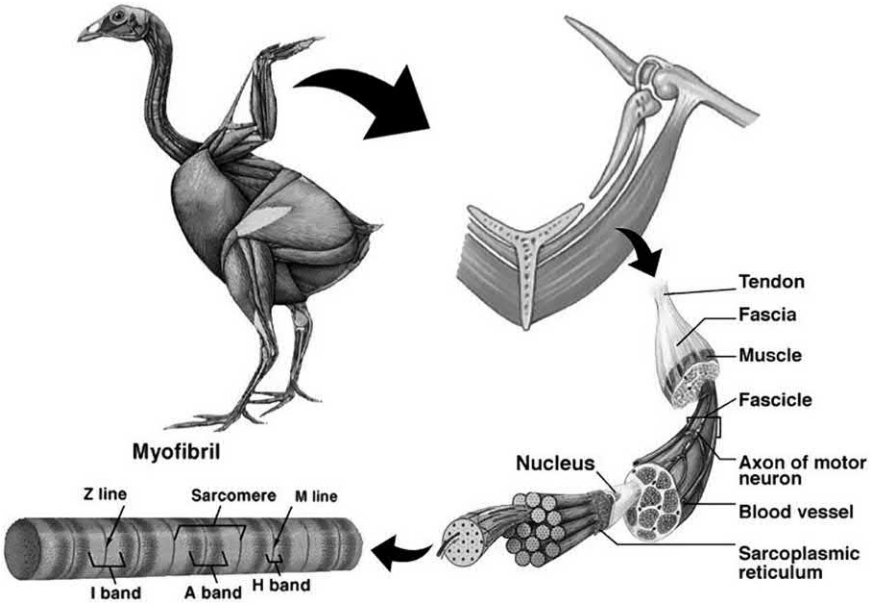

FIGURE 10.5 Broiler chicken skeletal muscle structure.

bundle is composed of small muscle fibers that are covered by a thinner layer of connective tissue called endomysium. Skeletal muscles have elongated fibers that are usually multinucleated, which seems to permit better control over these long cells. Each fiber consists of numerous myofibrils that have myofilaments inside them forming the sarcomeres. The overlapping between actin and myosin is called the A-band, and within the thin filaments is also the I-band. Sarcomeres are the distance between two Z-lines. During muscle contraction, the thick filaments slide toward the Z-line and shorten the sarcomere which the muscle moves.

10.3.1 Muscle Structure

Broiler muscle structure and physiology will be described to enable an understanding of their effect on meat quality characteristics and their effects on post-slaughter changes during rigor mortis, deboning, packaging, and storage. Muscle tissue represents the major edible part of the broiler that is important to both meat processors and consumers. Broiler skeletal muscle is a syncytium that is formed by a single nucleated mesodermal cell that undergoes terminal differentiation to form myoblasts, which then fuse to form multinucleated muscle fibers [10]. A muscle is composed of numerous muscle bundles covered by epimysium and separated from the others by a connective tissue layer called perimysium (Figure 10.5). The connective tissue provides structural organization, anchors the different components, and transmits the power generated by sarcomere contraction. Blood vessels supply energy with the nerves stimulating muscle movement. The muscle bundle is composed of smaller muscle fibers that are covered by a thinner layer of connective tissue called endomysium. Skeletal muscles have elongated fibers that are usually multinucleated, which seems to permit better control over these long cells. Each muscle fiber consists of numerous myofibrils that have myofilaments inside them forming the sarcomeres. The A-band represents the dark area in a stained muscle preparation which is the result of thin and thick filaments overlapping. The light band within the A-band area is called the H-zone (Figure 10.5). The area with only thin filaments is referred to as the I-band. Sarcomeres are the distance between the two Z-lines. Each sarcomere consists of one set of thick (myosin) filaments and two sets of thin (actin) filaments. During muscle contraction, the thin filaments are pulled in over the thick filaments toward the Z-line so that each sarcomere shortens and generates force [11]. The force to produce contraction is generated by the myosin cross-bridge; the head of myosin is the molecular motor. This has a hinged lever arm, to which two smaller proteins, the myosin light chains, are attached. Each cross-bridge is an independent force generator, which interacts with a thin filament and pulls it towards the center of the sarcomere [10]. The cross-bridge then detaches from the thin filament and has to be reprised by adenosine triphosphate before it can go through another cycle of force generation. The intrinsic velocity of contraction of muscle fibers to the specific activity of their myosin ATPasevia, the enzymatic activity of myosin, was discussed by Baranay [12]. The rate of cross-bridge consumption of ATP depends on the type of muscle fiber and the kind of activity for which it is adapted [13, 14].

10.3.2 Muscle Fiber Type

Broiler skeletal muscle fibers, similar to mammalian muscle fibers, can also be divided into white, red, and intermediate muscle fiber types. Broiler white meat refers to breast muscle, white and dark meat refers to the leg muscle. This is based on the color of the muscle, which is related to the proportion of red and white muscle fiber types. Most broiler chicken muscles contain a mixture of red and white fibers. Red, white,

TABLE 10.1
Relative Comparisons between Red and White Muscle Fibers in Poultry

Characteristic	Red Fiber	White Fiber
Myoglobin (conc.)	High	Low
Color	Red	White
Contraction speed	Slow	Fast
Mitochondria (number)	High	Low
Glycogen content	Low	High
Lipid content	High	Low
Oxidative metabolism	High	Low

Source: Barbut [49].

and intermediate muscle fiber types have different physiological functions and therefore have different proportions of certain sub-structures and metabolic rates (Table 10.1). Muscles with a high proportion of red muscle fibers (slow oxidative type 1; high myoglobin) are used for activities such as supporting the skeleton in an upright position due to their unique metabolism (less easily fatigued). A constant oxygen supply is important, and, together with a high proportion of enzymes involved in oxidative metabolism, the fibers can function for extended periods of time. Broiler red muscle fibers contract at a slower rate with the capacity to operate for a longer period of time due to the presence of mitochondria and lipid. The red muscle fiber type has a form of myosin which hydrolyzes ATP slowly resulting in a slow cross-bridge cycle. They are more efficient and more economical for producing slow repetitive movements and sustaining isometric force but not for generating power. The red muscle fibers are particularly numerous in postural muscles, which are activated virtually all the time during standing, walking, and running [15]. In broiler chicken there is another type of slow-twitch fiber, referred to as slow tonic, which is found in muscles such as the anterior latissimus dorsi [10]. This muscle holds the wings back against the body and is, therefore, contracted most of the time with very little expenditure of ATP [16]. Broiler white muscle fibers have less myoglobin with low oxidative activity (Table 10.2).

TABLE 10.2
Total Heme, Myoglobin, and Hemoglobin Content in Chicken Muscles

Muscle	Number	Total Heme	Hemoglobin	Myoglobin
Adductor	8	1.39 ± 0.31^{a}	0.83 ± 0.21^{a}	0.56 ± 0.17^{a}
Pectineus	8	0.10 ± 0.04^{d}	0.09 ± 0.04^{c}	0.01 ± 0.00^{b}
Sartorius	6	0.79 ± 0.12^{b}	0.67 ± 0.11^{a}	0.12 ± 0.02^{c}
Pectoralis	10	0.24 ± 0.04^{c}	0.24 ± 0.04^{c}	ND

Source: Kranen et al. [80].

[a–c] Per parameter, mean with no common superscript differ significantly as analyzed by t test ($P < 0.05$). Values are means ± SD of the numbers (n) of sample indicated. ND= not detectable.

The white muscle fiber type is adapted for high-power output over a short period of time (fast, glycolytic or type II2B), and the intermediate muscle fiber type is adapted for high-power output over a longer period of time (fast, oxidative, glycolytic or type II2A). Glycolytic metabolism, which predominates in white fibers, can occur with or without oxygen. Muscles with a relatively high content of white fibers show lower capillary density. Both types IIB and IIA muscle fiber contain myosin and other contractile proteins that produce a fast cross-bridge cycle time and develop force rapidly [10]. The type IIA fibers contain more mitochondria and have a more oxidative metabolism than the type IIB fibers and are capable of sustaining high-power output over a reasonably long period. White fibers contract more rapidly and in shorter bursts compared to red fibers, and they are more easily fatigued.

10.3.3 Muscle Protein

Broiler muscle proteins represent 18–20% of lean muscle weight with 75% water and 3–5% fat. Broiler muscles contain more than 50 types of different proteins. Table 10.3 shows the three major proteins based on their water and salt solubility [17]. The largest proportion of myofibrillar proteins in broiler muscle is myosin (45%). This is an elongated, rod-shaped

TABLE 10.3
The Major Protein Portions in an Average Muscle Divided into Three Groups According to Their Solubility and % in the Wet Muscle

Group	Protein	%	Description
Sarcoplasmic	Myoglobin	(5.5)	Sarcoplasmic proteins are distributed within the sarcoplasm fluid; it consists of myoglobin and enzymes and comprises about 30% of the muscle's protein.
	Hemoglobin	0.2	
	Cytochromes	0.6	
	Glycolytic enzymes	0.2	
		2.2	
	Creatine kinase	0.5	
Myofibrillar	Myosin	(11.5)	Myofibrillar proteins are the building blocks of muscle (contractile or cytoskeletal proteins), and actin (thin filament) and myosin (thick filament) make up the main portion of protein and comprise about 55% of the muscle proteins.
	Actin	5.5	
	Troptomysin	2.5	
	Troponin	0.6	
	G-protein	0.6	
	A-actinin	0.3	
	B-actinin	0.3	
		0.3	
Stromal	Collagen	(2.0)	Stromal proteins (collagen and elastin) are neither water- nor salt-soluble and represent about 12% of muscle protein and form structural components including membranes that surround cells, muscle bundles.
	Elastin	1.0	
	Mitochondrial	0.05	
		0.95	

Source: Asghar et al. [17].

protein with a very high molecular weight (around 450,000 Daltons). The structure has two heavy chains (heads) and two light chains (tails), which can be separated when myosin is subjected to a specific proteolytic enzyme activity. Actin (thin filament) has a proportion of 2.5%, has a lower molecular weight of 42,000 Daltons, and consists of two chains of F-actin. There is one tropomyosin molecule (5% of total myofibrillar proteins) for every seven actin molecules. There are three troponins (C binds Ca; I inhibits ATP and T binds tropomyosin), which are located around the thin filament and constitute about 5% of the myofibrillar proteins.

10.3.4 Rigor Mortis

At postmortem, oxygen and nutrient supply to the muscles will stop and many homeostatic mechanisms are disrupted including the normal aerobic tricarboxylic acid cycle. Energy metabolism switches to an anaerobic pathway to provide the muscle with ATP [18]. When blood circulation stops, lactic acid accumulates in the muscle until stored glycogen is depleted (ultimate pH). In broiler chicken, the drop in muscle pH occurs quickly as it does in other livestock [18]. In some broilers, glycogen storage has been depleted prior to slaughter due to malnutrition, extended activity, or struggling, which results in low lactic acid production (high ultimate pH: dark, firm, and dry). Muscle pH and meat color have consistently been reported to be highly correlated, especially when wide ranges of meat color are examined [19]. This is especially true when referring to meat as being either pale, soft, and exudative (PSE-like) or dark, firm, and dry (DFD-like) in which case pH is strongly related to these extreme conditions. Higher muscle pH is associated with darker meat while lower muscle pH values are associated with lighter meat color. In the extremes, high-pH meat is often characterized as DFD and the lighter meat as being PSE. Both DFD and PSE meats have been associated with poor functional properties. However, the pH can drop very quickly while the carcass temperature is still high (>35°C). This results in so-called PSE meat [20]. The combination of high temperatures (>35°C) and low pH values causes muscle protein denaturation [21, 22]. The meat color is pale as a result of more light reflected from the loose muscle structure as compared to the tight structure of the high ultimate pH meat. Conditions before and during rigor can have major significant effects on broiler meat quality characteristics. These include maintaining an adequate temperature during the rigor mortis process to prevent shortening and/or toughening of the muscle. In broilers, the meat temperature should be above 15°C (18 ± 2°C) because the rigor process takes 13 hrs. Although a reduction in muscle temperature after slaughter is important to prevent microorganism growth, reducing the temperature too quickly to below 5°C can cause low meat tenderness in broiler meat [23]. Temperature reduction to sub-zero temperatures, prior to the completion of rigor mortis, results in a condition known as thaw rigor due to the release of excess calcium from the sarcoplasmic reticulum into the sarcoplasm [18, 21]. Cold shortening can also cause significant toughening and moisture loss problems. Increasing the muscle temperature above 50°C during the rigor process will also result in excessive shortening known as heat rigor. This is the consequence of rapid ATP and creatine phosphate depletion.

Rigor mortis follows the depletion of glycogen and other energy sources such as creatine phosphate from the muscle, and results in its temporary toughening. When the broiler muscle pH drops to below 6, the sarcoplasmic reticulum calcium cannot store calcium, resulting in more interaction between myosin and actin [23]. In the presence of ATP muscle, myosin and actin start to overlap and consequently become less extensible (onset of rigor mortis). When all the energy sources have been depleted, the actomyosin cross-bridges can no longer be separated and the muscle becomes inextensible. After aging, the muscle becomes flexible again as a result of the action of the proteolytic enzymes (calpains and cathepsins) that slowly break down the sarcomere components including the degradation of the myofibrils, the connective tissue, and degradation of individual proteins such as titin, nebulin, and desmin [22, 24].

10.4 MEAT QUALITY CHARACTERISTICS

The major broiler meat quality attributes are tenderness, juiciness, flavor, color, and functionality [19]. Quality evaluation of raw broiler meat and products is very important to the poultry industry as it helps to control product quality, design, and optimizing of processes. Shear, tension, and torsion can help to optimize formulations and predict the quality characteristics (hardness and chewiness) that will be perceived by the consumer. Sensory evaluations are more time consuming but provide more precise information and can also be used to evaluate flavor, aroma, and overall acceptability of the product.

Evaluating the tenderness parameters of a meat product is important for quality control operations and for optimizing ingredient use/processing conditions to consistently produce an acceptable product. A product that is either too tough or too soft will be unacceptable to the consumer. Texture evaluations are done by several tests including shear, penetration, compression, tension, and torsion. A significant relationship has been observed between ultimate pH (pHu) and dripping loss in broiler chickens [25–27]. However, it is unlikely that a difference in pHu entirely explains the difference in water-holding capacity (WHC), because the magnitude of difference for pHu was quite small.

10.4.1 Meat Tenderness

Tenderness is probably the single most critical quality factor associated with the consumer's ultimate satisfaction with a broiler meat product [19]. Broiler meat tenderness depends upon the maturity of the connective tissues, the contractile state of the myofibrillar proteins, and the chemical and physical changes occurring in the muscle as it becomes meat (rigor mortis). For example, birds that struggle before or during slaughter cause their muscles to deplete glycogen, and rigor

mortis forms much faster than normal. These muscles tend to be tough because energy sources are reduced in the live bird. High pre-slaughter stunning, high scalding temperatures, longer scalding times, and machine picking can also cause poultry meat to be tough. The myofibrillar protein impacts on ultimate meat tenderness are primarily a function of the biochemical predisposition of the muscle at the time of slaughter, the rate and severity of rigor mortis development, and the physical handling of the carcass and muscle during rigor development [19].

The tenderness of portioned or boneless cuts of broiler chicken is influenced by the time postmortem of the processing. Muscles that are deboned during the early postmortem still have energy available for contraction. When these muscles are removed from the carcass, they contract and become tough. To avoid this toughening, meat is usually "aged" for 6 to 24 hours before deboning. However, this is usually costly for the processor. When poultry is deboned early (0 to 2 hours post-mortem), 50 to 80% of the meat will be tough. This means that if the processor waits 6 hours before deboning, 70 to 80% of the poultry meat will be tender. It has been established that broiler meat must be allowed a postmortem aging period prior to cut-up and deboning, cooking, or freezing to avoid adverse toughening, especially in breast meat [28]. Fletcher [19] suggested that a minimum of 4-h aging is required to allow the breast muscles sufficient time to complete rigor development and to allow subsequent removal from the carcass without excessive toughening. The concept of accelerated processing is based on the rapid depletion of muscle glycogen and ATP stores postmortem. Once the muscle loses the ability to generate ATP, rigor contractions cease and the muscle can be released from skeletal restraint with less adverse toughening.

Broiler meat tenderness can be evaluated using a shear force device. It uses a blade/knife to cut the meat sample, whereas penetration tests use a flat/round probe. The values obtained are usually correlated with sensory analysis. A less tender cut of meat will show a higher shear/penetration value. The Warner Bratzler (WB) shear device employs a single blade to shear a core meat sample and provides values for peak force. Shear determination is usually evaluated on intact pieces or cores of sample large enough to ensure a representative sample. The Allo-Kramer shear device (AK) is routinely used by researchers and quality control personnel, and the same considerations regarding the size, muscle, fiber orientation, etc., have been noted for the AK as for the WB. The AK employs a cell consisting of 10 to 13 blades that is guided into a square box to shear a large sample.

Lyon and Lyon [29] showed the effect of broiler breast meat deboning time on meat tenderness using the WB and AK shear methods and correlated the results with a sensory panel (Table 10.4). The results showed that deboning time had a significant effect on the shear values of broiler meat samples using both methods. Both shearing procedures were sensitive enough to discriminate between the three deboning times. The sensory panel showed similar results and tenderness was highly correlated with both shear measurement methods. Juiciness was not significantly related to deboning time but related to tenderness acceptability, with high correlation between values obtained from both shear measurement methods. In the poultry industry WB values of $\leq$4.5 kg are preferred for deboned chicken breast meat sold to the consumer.

Penetration tests are commonly used for restructured products, i.e., those made from small, ground or flaked pieces of meat, and some emulsified meat products such as frankfurters using a small-diameter probe which descends into the product

TABLE 10.4
Warner-Brazler and Allo-Kramer Shear Values for Intact and Diced Cooked (80°C) Samples of Broiler Breasts Deboned at Three Postmortem (PM) Times and Sensory Values of Cooked Chicken

	Tenderness		Sensory		
Deboning Time	Warner-Bratzler[1] (WB)	Allo-Kramer (20-g-diced) (AK)	Juiciness[2]	Tenderness[3]	Acceptability[4]
(h PM)	(kg)	(kg/g)			
2	9.5 ± 3.9[a]	5.2 ± 1.0[a]	3.5 ± 1.3	2.5 ± 1.3[c]	2.0 ± 0.9[c]
6	4.7 ± 1.6[b]	3.4±0.8[b]	3.4 ± 1.2	3.8 ± 1.2[b]	2.6 ± 0.9[b]
24	3.2 ± 0.9[c]	2.2±0.2[c]	3.5 ± 1.3	5.1 ± 0.8[a]	3.0 ± 0.9[a]
Correlations (r values)					
With WB			0.06	–0.90	–0.92
With AK			0.00	–0.99	–0.93

Source: Lyon and Lyon [29].

[a–c] Values within a column with no common superscript differ significantly ($P < 0.05$). For tenderness, mean values are averages of 66 observations (22 birds × 3 replications) for each deboning time. For sensory, 22 panelists × 3 replications. One bench-top Warner-Bratzler device was used to shear a 1.9 cm-wide intact strip. 2 Category scales 1 = very dry, tough, to 6 = very juicy, tender. 4 Category scales: 1 = poor to 5 = excellent.

at a constant rate. The resistance to puncture of the processed product is determined while obtaining a force-deformation curve, and the results are usually used to compare relative toughness.

10.4.2 Electrical Stunning and Electrical Stimulation

Electrical stunning has been shown to improve broiler meat tenderness and alter postmortem rigor and enhance meat quality [61]. Stunned broiler had higher ATP, lower lactate, and higher pH values than non-stunned broiler [30]. Stunning of broiler chicken indirectly delayed postmortem glycolysis (onset of rigor mortis) primarily through a suspension of antimortem struggle than through any direct effects of electrical stunning on muscle metabolism [31, 32]. High-current stunning resulted in a greater effect on early rigor delay than low-voltage stunning [19]. There are differences in muscle glycogen and pH during rigor development but these do not affect ultimate muscle pH, tenderness, or meat quality parameters.

Electrical stimulation is an optional technique to be used within 20 to 30 minutes postmortem on broiler chickens to trigger muscle contraction by stimulating the nerve system to speed up the onset rigor mortis [18, 33, 34]. Electrical stimulation involves passing an electric current through the carcass to overcome some of the problems associated with pre-rigor deboning that might be encountered during rapid chilling. Muscle contractions during electrical stimulation deplete the glycogen within the muscle and cause a rapid onset of the rigor mortis process. Electrical stimulation can rupture muscle fibers and cause physical damage to the sarcomere structure by tearing off some of the sarcomeres, which leads to the improvement of meat tenderness [35]. Electrical stimulation can accelerate ATP depletion of broiler carcasses by falling at 3.5 h post-mortem without the risk of getting tough meat and allowing deboning at an earlier stage. Electrical stimulation can also prevent or minimize cold shortening problems [34]. There are large variations in voltage, frequency, current, method, and time of application used among different researchers [36], which may result in variable and inconclusive results. Generally, a large number of broiler-processing plants are installing electrical stimulation equipment in order to shorten the processing time so that tender breast meat fillets can be harvested immediately after chilling. In countries where deboning is traditionally done 1 h after bleeding, electrical stimulation can help to reduce meat toughening. The carcass is suspended from a moving shackle line and is touched with a metal plate through which a current is passed. Usually the equipment can deliver up to 500 V (AC) and can be set to pulse at 0.2 to 2.0 sec intervals.

A combination of high-temperature conditioning and electrical stimulation could be used to produce early-deboned breast meat. Sams [37] reported that high-temperature conditioning or electrical stimulation had no individual effects on early-deboned broiler breast meat, but when combined they resulted in more tender breast meat. Walker et al. [38] concluded that only high-voltage electrical stimulation with muscle tension had a significant positive effect on muscle tenderness. In this respect, the meat temperature should be above 15°C (18 ± 2°C) because the rigor process takes 13 h when electrical stimulation is applied. If no electrical stimulation is used, the range is longer and can be 38 h; poultry carcass chilling in modern processing plants starts about 30 to 60 min after slaughter and reaches 15°C when rigor is completed or almost completed. It has been shown that electrical stimulation accelerates rigor development while electrical stunning delays rigor development. The electrical stimulation of broiler chicken is most effective following high-current stunning but had little effect following low-voltage stunning.

10.4.3 Meat Flavor

Flavor is one of the most important factors in determining the acceptability of broiler meat. Intramuscular fat and inosinic acid are key factors that influence the flavor of broiler meat [39]. A significant amount of meat flavor develops during cooking via complex reactions between natural compounds present in raw meat [40, 41]. This is evidenced by the aroma of cooked meat, which is completely different from that of raw meat. Many of the compounds produced during cooking have relatively high odor thresholds and present little contribution to the overall aroma and flavor. Gas chromatography and mass spectrometry can be used to identify major compounds of importance for flavor and the lower concentration compounds with which they interact. Meat flavor is a combination of taste and smell, which are perceived by the taste buds and olfactory receptors in the nose, respectively [42]. Meat taste and smell are affected by numerous factors such as the quantity and ratio of different flavor compounds, fat content, and temperature. Taste is perceived by sensors on the tongue that are capable of detecting four major tastes: salty, sweet, sour/acid, and bitter [41].

Cooking meat generates hundreds of volatile compounds. In this respect, Farmer [42] indicated about 500 volatile components in chicken meat. The precursors may include amino acids, reducing and phosphorylated sugars, lipids, and thiamine. Most volatile compounds are present in concentrations below their taste threshold, which suggests that synergistic effects are important in taste perception.

Using diluted aroma extracts (volatile compounds) that can be detected by humans as an assessment method for broiler meat flavor, gas chromatography was used to identify many individual volatile compounds. Discrepancies in the values reported by different researchers may be due to the complexity of sensory perception and the effect of different methods used for extraction, sample preparation, and method of assessment. The effect of the major volatile compounds can be evaluated by entirely removing them from the food or supplementing the food with more of an individual compound [40, 43]. A combination of water-soluble compounds in a cooked broiler chicken extract with amino acids, ATP metabolites, and inorganic ions to simulate the sensory properties of the extract was carried out by Fujimura et al. [43]. They found a glutamic acid (salty tastes), inosine monophosphate (sweetness),

and potassium ions (salty, bitter, or sweet sensation) were the major compounds. During cooking, a change in the concentrations of reducing sugars, amino acids, and nucleotides was observed. This affects the taste and aroma of poultry meat. Ribose appeared to be very important in increasing aroma in roasted broiler chicken and the researchers concluded that the change in odor was probably due to elevated concentrations of compounds such as 2-furanmethanethiol, 2-methyl-3 furanthiol, and 3-methylthiopropanol [40].

The list of the most important volatile compounds in broiler meat is grouped into furanthiols and disulfides, sulfur-containing compounds, aldehydes, ketones, and lactones, heterocyclic (Table 10.5). The latter author concluded that the combination of these compounds can be responsible for providing the typical aroma of a cooked broiler chicken. The important compounds responsible for cooked broiler chicken aroma differ from other meats in that 2-methyl-3-furyl disulfide, methional, and phenylacetaldehyde are less important and certain lipid oxidation byproducts such as trans-2,4-decadienal and trans-undecenal are more important [44]. They concluded that this difference may be related to the higher concentrations of linoleic acid in broiler chicken than meat from other species. It should also be mentioned that cooking methods have a strong effect on flavor and aroma. For instance, fried meat has a different aroma than boiled meat.

The chemical reactions important for flavor and aroma production can be categorized into three main groups: Maillard reactions, lipid oxidation, and degradation of thiamine. A Maillard reaction is the reaction of one or more amino acids with reducing sugar, which can result in many volatile products. Lipid oxidation contributes to a desirable flavor and aroma, but can also cause rancidity in cooked broiler chicken meat. Oxidation occurs at ambient temperature by endogenous enzymes, which result in the production of negative flavor and aroma described as rancid or cardboard-like. The information in Table 10.5 shows ten compounds that result from thermal oxidation, such as 1-Octen-3-one, trans-2-nonenal, trans trans-2,4-nonadienal, and trans trans-2,4-decadienal. The most reactive lipids in broiler meat are the polyunsaturated fats, followed by monounsaturated and saturated lipids, which contribute to the unpleasant odor of reheated cooked broiler meat (warmed-over flavor).

Thiamine (vitamin B1) degradation can produce sulfur- and nitrogen-containing end products that result from the breakdown of the vitamin's bicyclic structure. The 2-methyl-3-furanthiol has a potent aroma, which is responsible for a

TABLE 10.5
List of Some of the Major Compounds Contributing to Odor of Cooked Broiler Meat

Compound	Odor Character	Compound	Odor Character
Furanthiols and disulfides		2-undeccenal	Tallowy, sweet
Bis (2-methyl-3-furyl) disulfide	Meaty, roasted	y-decalactone	Peach-like
2-methyl-3-ifcanthiol	Meaty, sweet	y-dodecalactone	Tallowy, fruity
2,5-dimethyl-furanthiol	Meaty	**Heterocyclic Compounds**	
2-funinmethanethiol	Roasted	2-formyl-5-methyl thiophene	Sulfurous
2-methyl-3-(methylthio) furan	Meaty, sweet	Trimethylthiazole	Earthy
2-methyl-3-(ethylthio) furan	Meaty	2-acetyl-2-thiazoline	Roasted
2-methyl-3-methyldithiofuran	Meaty, sweet	2,5(6)-dimethyl-pyrazine	Coffee, roasted
Sulfur Containing		2,3-dimethyl-pyrazine	Meaty, roasted
3-mercapto-2-pentanone	Sulfurous	2-ethy1-3,5-dimethyl-pyrazine	Roasted
Dimethyltrisulfide	Grassy, metallic	3,5(2)-diethyl-2(6)-methyl-pyrazine	Sweet, roasted
Hydrogen sulfide	Sulfurous, eggy	2-acetyl-pyrroline	Popcorn
Methional	Cooked potatoes	**Other**	Caramel
Aldehydes, Ketones, and Lactones		2,3-butanedione	
1-octen-3-one	Mushrooms	B-ionone	Violets
trans-2-nonenal	Tallowy, fatty	14-methyl-pentadecanal	Fatty, tallowy, train-oil
Nonanal	Tallowy, green	14-methyl-hexadecanal	Fatty, tallowy, orange-like
trans, trans-2,4-nonadienal	Fatty	15-methyl-hexadecanal	Fatty, tallowy
Decanal	Green, aldehyde	4-methylphenol	Phenolic
trans, trans-2,4-decadienal (and an isomer)	Fatty, tallowy		

Source: FAO [42]

"meaty" aroma and flavor in broiler meat [41, 44]. The compound can be formed by thiamine degradation or by a reaction between cysteine and ribose. 2-furanmethanethiol can also be formed by thiamine degradation, and provides a "roasted" aroma in broiler meat. Salama [45] found that conventional heating provides a preferable flavor for leg and breast broiler meat than microwave heating. This may be due to how rapid microwave heating is (rapid change of water molecule polarity throughout the product), which does not allow enough time for odor and flavor development. It has been reported that adding sodium chloride to broiler meat, prior to cooking, will increase the flavor rating under both conventional and microwave heating. The amounts of volatile compounds produced increase as cooking temperature increases from 60 to 80°C [46]. Increasing cooking temperatures from 60 to 70°C can increase the rate of the chemical reactions as well as the release of free amino acids and other precursors and quantities of lipid oxidation byproducts. The amounts of 2-, 3-butanedione, and dimethyl disulfide increased at an almost constant rate between 60 and 80°C [46].

In general, the characteristic flavor of broiler meat is derived from the presence and concentration of various water-soluble and volatile aroma compounds. The concentrations and interactions between these compounds can be affected by age and diet, as well as by processing factors such as evisceration time, chilling rate, storage, and cooking method [41, 42, 47]. Touraille et al. [48] found no flavor differences between sexes until broilers reach sexual maturity. Large dietary changes are required to produce a small change in broiler meat flavor [47]. However, a small amount of feed, such as oxidized fish oil, can induce a fishy aroma in broiler meat [49]. Supplemental vitamin E has been shown to enhance the shelf life of stored poultry meat by retarding lipid oxidation and off-flavor formation during prolonged fresh/frozen storage [50].

10.4.4 Meat Color

Of all the quality attributes, color is the most critical for the selection of broiler products because consumers associate it with the product's freshness, and consequently decide whether or not to buy the product based on their opinion of its attractiveness [19]. Broiler meat is unique because it can be sold with or without skin. Skin color is the most critical characteristic for the marketing of fresh whole broiler or parts [51]. Broiler is also the only species known to have muscles that have extremes in color (white and dark meat). Breast meat is expected to have a pale pink color, while thigh and leg meat are expected to be dark red. The major contributing factors to broiler meat color are myoglobin content, chemical state of the heme structure, and meat pH [19]. Myoglobin content has been shown to be primarily related to species, muscle, and age of the bird. Muscle pH has been shown to be primarily related to the biochemical state of the muscle at the time of slaughter and following rigor mortis development. A significant correlation between L^* of the broiler meat and its pHu has been reported [4, 26]. The redness of chicken breast meat has been shown to positively correlate with myoglobin content [52]. This is lower in glycolytic than in oxidative myofibers [53]. A significant increase in L^*, a^*, and b^* was observed between 4 and 10 days post-slaughter, which could result from myosin degradation [27, 54]. This variation may be related to the chemical changes in muscle myoglobin pigment, which is predominantly converted into purple reduced myoglobin and brown metmyoglobin during the first several days postmortem [55]. This may be due to muscle fiber type; the major muscle of chickens is almost a completely white fast-twitch muscle [56]. Lightness appeared to be highly correlated with pHu.

Variations in raw breast meat color are sufficient to cause variations in cooked product color [57]. Broiler meat color is affected by factors such as bird age, sex, strain, diet, intramuscular fat, moisture content, preslaughter conditions, and the presence of the muscle pigments myoglobin and hemoglobin [58]. Discoloration of broiler meat can be related to the amount of these pigments present in the meat, the chemical state of the pigments, or the way in which light is reflected off of the meat [59]. The discoloration can occur in an entire muscle, or be limited to a specific area, such as a bruise or a broken blood vessel. When an entire muscle is discolored, it is most probably the breast muscle. This occurs because breast muscle accounts for a large portion of the live weight, it is more sensitive to factors that contribute to discoloration, and the already light appearance makes small changes in color more noticeable. Extreme environmental temperatures or stress due to bird handling and before processing can cause broiler breast meat to be discolored. The extent of the discoloration is related to each bird's individual response to the conditions.

Another major cause of poultry meat discoloration is the nature and reactions of the major pigment, myoglobin, as well as the effects of nitrates and nitrites, ovens and gasses, hemachromes, and cytochrome C reactions on final meat color [59, 60]. The poultry industry generally tries to identify where, how, and when the injuries causing discoloration take place, but this is often difficult to determine. The color of the bruise, the amount of "blood" present, and the extent of the "blood clot" formation in the affected area are good indicators of the age of the injury and may give some clues as to its origin. Electrical stunning at high currents (greater than 100 mA) was shown to increase blood spots in broiler breast meat [61]. A bruise will vary in appearance from a fresh, "bloody" red color with no clotting minutes after the injury to a normal flesh color 120 hours later (Table 10.6). The amount of "blood" present and the extent of clot formation are useful in distinguishing if the injury occurred during catching/transportation or during processing. Injuries that occur in the farm are usually magnified by processing plant equipment or handling conditions in the plant. Because bruises are a major source of condemnation and downgrading [62] efforts to reduce their incidence have been identified.

The relationship between muscle type and bird age and its effect on meat myoglobin content and color was studied by Miller [63]. He found that white meat from 8-week-old broiler had the lowest myoglobin content (0.01 mg myoglobin/g meat) compared with 26-week-old broiler white meat (0.10 mg/g),

TABLE 10.6
Broiler Meat Color Changes in a Bruise over Time for Broiler Muscle

Age of Bruise	Color of Bruise
2 minutes	Red
12 hours	Dark red-purple
24 hours	Light green-purple
36 hours	Yellow-green-purple
48 hours	Yellow-green (orange)
72 hours	Yellow-orange
96 hours	Slight yellow
120 hours	Normal, flesh color

Source: Gregory [54].

8-week-old broiler dark meat (0.40 mg/g), and 26-week-old broiler dark meat (1.50 mg/g). In thigh meat, color parameters were affected by genetic strain [64]. Low pH values affect meat protein biochemistry, resulting in higher lightness and redness and lower yellowness. This was confirmed by the relation between pH and color parameters [64]. The effect of genotype on color parameters was reported by several authors [65–67]. This apparent inconsistency may be due to bird movement, as birds reared in free-range systems graze and perform more activity, changing muscle metabolism, with higher development of red muscle fibers [68].

10.4.5 Sensory Analysis

Sensory analysis of broiler meat includes different parameters based on the senses of taste (salty, sweet, sour, and bitter), smell, touch (tenderness, mouthfeel, and moisture level), sight (color, shape), and hearing (crunchy). The field of sensory analysis has scientifically developed over the years to become a recognized discipline in food science for food evaluation. Trained sensory professionals work in areas such as quality control, product development, tenderness and flavor research, and ingredient and process modification. Good sensory evaluation teams can ensure that successful products with desirable sensory attributes reach the market [69]. The sensory evaluation technique is a scientific method used to evoke measure, analyze, and interpret human responses to products via the senses of taste, touch, smell, and sight. There is a set of guidelines that should be followed in preparing and serving the samples under controlled conditions so that bias is minimized. In general, sensory evaluation techniques involve quantitative science in collecting data to establish legitimate and specific relationships between product characteristics and human perception. However, there can be many sources of variation that cannot be completely controlled during the test (previous food eaten, physiological sensitivity to sensory stimulation, mood, past history, and familiarity with similar products). Therefore, some screening should take place to eliminate participants with taste and color vision insensitivity [70].

10.5 MEAT COMPOSITION

Broiler meat has been an important part of the human diet for thousands of years with a significant role in human nutrition due to its high -quality proteins and essential amino acids, lipids and essential fatty acids, vitamins, and minerals [71]. It has also desirable sensory parameters with positive aspects for human health (low fat and high protein) and an acceptable price [72]. Overall, the dietary contribution of broiler meat is dependent on culture, availability, and nutritional value. The presence of skin on a poultry meat cut will increase the fat level of the portion because skin includes subcutaneous fat, and as fat content increases, moisture content decreases (Table 10.7). Overall, broiler meat consumers can obtain a very lean product by removing the skin because most of the fat is deposited subcutaneously rather than intramuscularly. Therefore, the emphasis has been on a better body composition, with higher breast meat yield and lower abdominal fat. This focus responds to the consumer desire for healthier meat, and to the evolution of the market through a rising demand for portioned and processed products [73]. The profitability of broiler production is therefore largely determined by the possibility of increasing the proportion of prime cuts in the carcass, mainly breast meat, and by reducing fat. Based on a 100 g lean fillet portion, lean broiler breast contains relatively higher protein % (>10% compared to beef and lamb), monounsaturated fatty acids (>20% compared to beef and tuna), and polyunsaturated fatty acids (>30% compared to beef, lamb, and pork) [74]. The same author stated that lean breast of chicken also provided relatively more selenium (>25%) than beef, lamb, and pork, and more manganese (>90%) than lamb and relatively more vitamin E (α tocopherol) than lamb and beef (>40%). Differences in nutritional value between broiler chicken and other meat animals may be related to the ability of the chicken digestive system to take up nutrients from feeds and deposit them in the meat. It has been shown that the body composition of broiler chicken varies with age, gender, and bird strain [75]). As a broiler chicken grows the composition of its carcass changes [76] and fat deposits increase [75]. Ultimately the nutrient composition of broiler is a response to the diet that humans consume, particularly in the early stages of life [76, 77]. Knowing the nutritional composition of the product is central to communicating ways in which chicken may form part of a healthy diet.

Table 10.7 shows that the white broiler meat contained more protein than dark meat (20.3% vs. 16.7% with skin and 23.2% vs 21.1% without skin, respectively). When the skin is removed, the fat level in broiler meat drops from about 11.1 to 1.6% in white meat and from 18.3 to 4.3% in the dark portion. The effect of cooking methods on the nutrient composition of raw meat with skin on is presented in Table 10.8. Stewing, frying, and roasting methods result in the highest protein content due to loss of moisture and fat during cooking. Stewing reduces cooking loss (moisture) compared to roasting. This is due to more protein denaturation during the cooking process which results in a lower water-holding capacity. The cholesterol level remains similar to that of the roasting

TABLE 10.7
Composition and Nutritional Value of Broiler Meat

Meat	Skin	Moisture%	Protein%	Fat%	Calcium (mg)	Iron (mg)	Calories (kcal)
White	+	68.6	20.3	11.1	0.86	0.80	186
	–	74.9	23.2	1.60	0.98	0.70	114
Dark	+	65.4	16.7	18.3	0.76	1.00	237
	–	75.9	20.1	4.30	0.94	1.00	125

Source: USDA [81].
Expressed on 100 grams portion of meat with/without skin.

method because vegetable oil used for frying does not contain cholesterol.

10.5.1 Comparing Broiler Meat with Other Species

The high-value cuts of chicken, lamb, beef, pork, and fish were compared (Table 10.9). Chicken meat appears to be one of the highest protein sources of the "traditional" meats though the equal second when compared with fish. Similarly, the polyunsaturated fatty acid levels in chicken meat are also higher than for all other high-value cuts of meat; however the proportion of each of the fatty acids to the total fat profile should be considered as in Table 10.9, which shows tuna fish clearly having a higher proportion of polyunsaturated fatty acids. Overall, however, chicken meat appears to have the largest amount of monounsaturated fatty acids than all other meats used as a comparison in this report (Table 10.9). These nutrient levels indicated the ability of broilers to utilize the nutrients from their feed more effectively than other animals [78, 82].

Chicken meat appears to have higher values in a number of micronutrients than other raw meats, except fish. The levels of magnesium, calcium, selenium, manganese (Table 10.9), and phosphorus are all greater than those found in the highest-value cuts of beef, lamb, and pork. Values for iron and zinc are not high. Chicken meat also appears to contain greater amounts of vitamin A than beef and pork, more vitamin E than all traditional meat sources (excluding fish), more niacin than lamb and pork, more thiamin than lamb and beef, and more vitamin B_{12} than pork. These reports should be considered with caution as the data are from different sources in Table 10.9 and the animals quite possibly of varying ages, genders, feeds, and living conditions, and differences do not necessarily reflect significance in terms of recommended dietary intakes.

10.6 POSTHARVEST PORTIONS AND DEBONING

Increasing market demand for deboned broiler meat chicken has resulted in the development of mechanical cutting up and deboning operations equipment. Various cutting setups have been developed for the broiler meat processor, ranging from manual deboning to fully automated equipment to cut and debone the whole carcass. Many classification systems for the cutting of broiler carcass are used due to various demands from consumers in different countries. Consumers today have the option of purchasing a whole chicken fresh or frozen or cut up carcass parts including wings, breast fillets, drum sticks, and thighs. Depending on the market, broiler chicken can be sold alive, as an eviscerated whole carcass, the carcass split into halves or quarters, or as separate cuts with or without bones and skin. Although there are many similarities between different systems, each is designed to serve a specific market [49]; standard classification cutting systems are discussed below:

1. Live broiler chicken meat derived from slaughtering and dressing broiler chicken carcasses.
2. Dressed broiler chicken from the carcass after the feathers, head, the feet, and the carcass have been eviscerated.
3. Broiler chicken half carcass from two approximately equal portions of a dressed broiler carcass.
4. Front quarter from the front portion of a broiler half carcass obtained by cutting behind and parallel to the rib cage.
5. Hindquarter: meat from the posterior portion of a broiler chicken carcass which is separated from the front quarter.
6. Wings separated from the shoulder joint.
7. The drumstick is the distal portion of the leg, which is separated from the leg by a straight cut through the knee joint.
8. The thigh is the proximal portion of the leg, which is separated from the whole broiler carcass by cutting at the hip joint and from the drumstick by a straight cut through the knee joint.
9. The full breast is the portion of the whole broiler carcass which is separated from the wing cutting through the shoulder joint, from the back by cutting through the ribs at the junction of the vertebral ribs, and from the hind quarter by cutting behind the rib cage.
10. The half breast is one of the two equal portions of the breast separated by cutting through the breast bone along the median line.

TABLE 10.8
Effect of Three Different Cooking Methods on the Nutritional Composition of Light Chicken Meat with Skin

	Mean Values in 100 g, Edible Portion			
Nutrients	**Raw**	**Fried**	**Roasted**	**Stewed**
Proximate				
Water (g)	68.60	49.40	62.50	53.01
Energy (kcal)	186	289	190	285
Protein (g)	20.27	22.55	20.37	26.88
Fat (g)	11.07	17.35	11.38	18.87
Carbohydrate (total)	0	9.5	0	0
Minerals				
Calcium (mg)	11	18	10	11
Iron (mg)	0.79	1.26	1.07	1.16
Magnesium (mg)	23	18	17	17
Phosphorus (mg)	163	132	155	153
Potassium (mg)	204	157	180	155
Sodium (mg)	65	250	75	62
Zinc (mg)	0.93	1.46	1.23	1.50
Vitamins				
Thiamin (mg)	0.059	0.98	0.05	0.08
Riboflavin (mg)	0.086	0.161	0.120	0.200
Niacin (mg)	8.908	5.987	6.305	4.928
Vitamin B_6 (mg)	0.480	0.264	0.290	0.212
Folacin (mcg)	4	21	4	4
Vitamin B_{12} (mcg)	0.34	0.24	0.23	0.20
Vitamin A (IU)	99	79	71	33
Fat (g)				
Total saturated fatty acids	3.91	4.00	3.20	4.34
Lauric acid (C12:0)	0.01	0.01	0.01	0.01
Myristic acid (C14:0)	0.09	0.09	0.09	0.08
Palmitic acid (C16:0)	2.33	2.76	2.25	2.07
Stearic acid (C18:0)	0.63	1.2	0.62	0.56
Total monounsaturated FAs	4.52	6.02	4.59	6.11
Palmitoleic acid (C16:1)	0.60	0.45	0.57	0.53
Oleic acid (C18:1 n-9)	3.74	5.79	3.51	3.23
20:1	0.12	0.09	0.12	0.11
	2.34	3.48	2.48	3.59
Total polyunsaturated fatty acids				
Linoleic acid (C18.2n6c)	2.07	3.24	1.98	1.83
Alpha Linolenic acid (C18:3n3)	0.10	0.18	0.09	0.08
18:4	-	-	-	-
Archidonix acid (C20:4n6)	0.06	0.07	0.09	0.08
20:5	0.01	0.01	0.01	0.01
22.5	0.01	0.01	0.02	0.02
22:6	0.02	0.02	0.03	0.03
Cholesterol (mg)	67	84	84	74
Amino acids (g)				
Tryptophan	0.227	0.268	0.326	0.294
Threonine	0.839	0.963	1.202	1.084
Isoleucine	1.015	1.171	1.458	1.316
Leucine	1.477	1.723	2.119	1.91
Lysine	1.654	1.841	2.374	2.142
Methionine	0.541	0.616	0.776	0.699
Cystine	0.270	0.326	0.385	0.347
Phenylalanine	0.788	0.938	1.130	1.019
Tyrosine	0.665	0.762	0.940	0.848
Valine	0.985	1.17	1.412	1.273
Arginine	1.268	1.445	1.811	1.629
Histidine	0.597	0.682	0.858	0.774
Alanine	1.177	1.334	1.679	1.509
Aspartic acid	1.807	2.040	2.587	2.330
Glutamic acid	2.967	3.750	4.254	3.835
Glycine	1.291	1.466	1.823	1.629
Proline	0.973	1.238	1.381	1.238
Serine	0.714	0.869	1.021	0.919

Source: USDA [81].

11. The breast fillet is a round, elongated fusiform muscle.
12. The back is the portion of the whole carcass back which is separated from the neck by cutting of the shoulder joint.
13. The neck is the portion of the broiler chicken carcass obtained by cutting near the shoulder joint.
14. Ground broiler meat is a fresh, boneless, with or without skin comminuted meat that has a fat content identified by one of the following terms:
    a. Regular contains approximately 30%.
    b. Medium contains approximately 23%.
    c. Lean contains approximately 17%.
    d. Extra lean contains approximately 10%.

Traditional manual cutting and deboning of broiler chicken has been exercised for thousands of years and can still be observed in small-scale operations in many countries. Manual deboning is usually done on a cutting board. It can be done by cutting the anterior ends of the pectoralis and pulling it away from the bone. This will leave a small portion of meat attached to both sides of the sternum from which they can be manually pulled. The thigh cut is separated by cutting through the femur-pelvis joint. The leg portion can be separated into the drumstick and thigh by cutting through the femora-tibial joint. Boneless thigh meat can be obtained by removing the femur bone as well as the major ligaments.

Automated high-speed cutting and deboning equipment has become very popular with the increased demand for boneless broiler chicken meat. The advanced system can debone 3,600 breast caps/hr. The design of automated deboning

TABLE 10.9
Comparison of Available Nutrient Values for High-Value Cuts of Chicken, Lamb, Beef, Pork, and Fish Highlighting Nutrient for Which Chicken Meat Has a Greater Percentage

	Meat								
	Chicken	Lamb		Beef		Pork		Tuna	
	Breast	Tenderloin	% Difference	Fillet	% Difference	Fillet	% Difference	N/A	% Difference
Water (g)	74.7	73.9	1.07	75.0	–0.40	74.4	0.40	71.0	4.95
Energy (kJ)	438	485	–10.9	490	–12.0	458	–4.7	435	0.56
Fat (g)	1.60	4.00	–150	3.0	–87.5	2.30	–43.8	1.00	37.5
Protein (g)	22.3	19.8	11.01	22.3	–0.22	22.0	1.12	23.4	–5.17
SFA (g)	0.52	1.40	–166.8	1.10	–109.6	0.90	71.5	0.20	61.9
C14:0 (mg)	0.02	0.08	–316.7	0.09	–368.8	0.03	–56.3	0.01	47.9
C15:0 (mg)	0.00	0.01	–212.5	0.01	–212.5	----	-----	----	----
C16:0 (mg)	0.36	0.72	–101.8	0.64	–79.37	0.54	–51.4	0.16	55.2
C17:0 (mg)	0.01	0.05	–681.3	0.02	–212.5	0.01	–56.3	-----	-----
C18:0 (mg)	0.13	0.53	–303.9	0.32	–143.9	0.27	–105.8	0.05	61.9
MUFA (g)	0.74	1.80	–139.4	1.10	–46.3	1.00	–32.9	0.19	73.4
C16:1 (mg)	0.04	0.06	–0.44.2	0.09	–116.4	0.06	–44.2	0.03	27.9
C18:1 (mg)	0.65	1.74	–167.2	1.03	–58.2	0.88	–35.1	0.11	83.1
PUFA (g)	0.32	0.50	–58.6	0.30	4.82	0.30	4.82	0.30	4.82
C18:2 n-6 (mg)	0.22	0.24	–7.14	0.15	33.4	0.24	–7.14	-----	-----
C18:3 n-3 (mg)	0.01	0.04	–177.8	0.03	–108.3	0.02	–38.9	-----	-----
Cholesterol (mg)	59.0	70.0	–18.6	58.0	1.69	95	–61.0	45	23.7
Sodium (mg)	41.0	69.0	–68.3	57.0	–39.0	54	–31.7	37	9.8
Potassium (mg)	300	330	–10.0	380	–26.7	405	–35	444	–48
Magnesium (mg)	28.0	24.0	14.3	27.0	3.57	26	7.14	50	–78.6
Calcium (mg)	12.0	8.00	33.33	6.0	50.0	4	66.7	16	–33.3
Phosphorus (mg)	231	240	–3.90	230	0.43	237	–2.60	191	17.3
Iron (mg)	0.40	2.10	–425	2.20	–450	1.10	–175	0.70	–75
Zinc (mg)	0.70	2.90	–314.3	3.80	–442.9	1.70	–142.9	0.50	28.6
Selenium (mg)	21.4	10.0	53.3	12.0	43.9	15	29.9	37	–72.9
Copper (mg)	0.03	0.13	–306.3	0.15	–368.8	0.09	–181.3	0.06	–87.5
Manganese (mg)	1.64	0.02	98.8	----	----	----	----	0.02	99.1
Vitamin A IU	15.5	9.00	41.9	2.0	87.1	----	----	----	----
Vitamin E (mg)	2.20	0.50	77.3	0.9	59.1	0.3	86.4	0.50	77.3
Niacin acid (mg)	11.0	9.40	14.6	11.6	–5.5	10.2	7.3	14.3	–30
Riboflavin (mg)	0.19	0.27	–42.1	0.22	–15.8	0.19	0.00	0.05	73.7
Thiamin (mg)	0.11	0.09	18.2	0.05	54.6	0.82	–645.5	0.43	–290.9
Vitamin B12 (μg)	0.38	1.30	–242.1	1.9	–400	0.30	21.1	0.50	–31.6

Source: Probst [74].
Note: Abbreviations: SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids.

equipment represents a significant challenge to configurations with the same yield. This equipment should be capable of handling birds of different sizes; therefore it should look for flocks with low variation among birds. Recently, new modules have been developed for new end products such as tendon harvesters and cartilage harvesters, which were considered waste a few years ago.

The large demand for deboned broiler meat has resulted in the development of mechanical deboning equipment and the meat obtained is called mechanically deboned meat or mechanically separated meat. The deboning equipment is used for deboning the whole broiler carcass or meat from parts that would not yield a high-priced product which would justify the cost of hand deboning such as neck meat. Salvaging the meat after hand deponing can be of great economic importance, and the resulting minced meat is successfully used for making other further processed products (broiler frankfurters). There are three basic types of broiler chicken deboning on the international market.

1. A belt-drum system: the meat and bone particles are passed between a rubber belt and a perforated steel drum. The meat is squeezed through the holes of the drum while the harder bones and connective tissue

TABLE 10.10
Effect of Deboner Head Pressure on the Chemical Composition and Yield of Mechanically Deboned Whole Roasted Broiler Breasts with Bone and Skin vs. Composition of Broiler Meat Obtained by Hand-Deboned Meat Operation

Pressure (lbr/in²)	Moisture%	Protein%	Fat%	Ash%	Calcium ppm	Iron ppm	Palmedica %	Yield%
				Mechanically deboned				
40	69.82	20.65	8.13	1.05	582	10.03	23.0	45
75	70.37	20.76	7.88	1.04	534	11.70	22.8	44
120	70.28	20.10	8.47	1.12	568	10.60	24.7	42
150	71.05	20.68	6.78	1.23	764	17.85	27.3	82
				Hand deboned				
	73.20	23.67	3.10	0.94	162	6.25	20.1	--

Source: Barbut [49].

stay outside. The pressure on the belts can be adjusted, and sometimes pressure rollers are used to ensure an even distribution of tissue on the belt, which results in higher muscle structure integrity [79].

2. A rotating auger system resembles the inside of a conventional broiler meat grinder. First, bones are pushed through a bone cutter to reduce particle size, then the ground mixture is introduced into a screw-drive boning head where the material is processed. The meat then is squeezed out through holes in the perforated steel cylinder encasing the auger. Hole-size can be adjusted and is usually around 0.5 mm. Bone and connective tissue particles that cannot pass through the perforated cylinder are pushed forward and exit at the end [49].
3. A hydraulic press pushes the meat and bones against a perforated plate in a batch-type system. The bones can also be pre-cut prior to being introduced into a chamber. The material is forced against a stationary, slotted surface/plate by a hydraulic-powered ram piston that squeezes the soft meat tissue through the cylinder openings (1.0 to 1.5 mm). The residual bones and connective tissue are removed, and the meat is separated resulting in a minced/finely chopped product, which is suitable for frankfurters and bologna [49].

Table 10.10 presents the meat composition of deboning broiler meat under different pressures. The presence of skin on a poultry meat cut will increase the fat level of the portion because skin includes subcutaneous fat. Higher fat translates to a higher caloric value, but in general, broiler meat is considered a lean meat when compared to other red meats and has less saturated fat than other red meat species. Cooking methods affect nutrient composition in different ways. Stewing will increase protein content; roasting and frying also elevate protein content as moisture and fat are lost. Stewing reduces cooking losses, resulting in a more-moist product compared to roasting. Battering and breading (22% coating) of fried broiler meat raises the carbohydrate content from 0 to 9.5%, and the total fat content from 11 to 17%. The cholesterol level remains similar to that of the roasting method because vegetable oil used for frying does not contain cholesterol. Yields are also affected by the cooking method due to variations in cooking temperature, time, previous treatment (margination), and processing (freezing, chilling).

10.7 CONCLUSION

The poultry industry has become much more competitive over the past 50 years, and poultry is expected to become the number one meat source around the world. The dramatic changes in the poultry industry over the past 30 years, from a predominantly whole bird commodity to the modern highly diversified industry focused on cut-up, deboned meat and ready-to-eat processed products has also resulted in a change in consumers' quality expectations. Although the basic issues of color and tenderness are still critical quality issues, the specific and relative degree of importance has grown with the changes in market products. The most important aspect of poultry meat is its quality, a function of the combined effects of color, tenderness, and flavor. As the market continues to grow, quality parameters are expected to continue to evolve as well as the identification of new quality products. The market has shifted from traditional concepts of market-based quality, affecting price and preference, to a production-oriented definition of quality based on statistical control and product uniformity. Poultry processing affects meat quality by establishing the chemistry of the muscle constituents and their interactions within the muscle structure. The producer, processor, retailer, and consumer all have specific expectations for the quality attributes (color, water-holding capacity, and tenderness) of broiler chicken meat while the ultimate authority will always be the consumer.

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11 Postharvest Handling of Milk

Nejib Guizani and Zaher Al-Attabi

CONTENTS

11.1 INTRODUCTION

Milk is a normal secretion of the mammary glands of female mammals. The US Public Health Service defines milk as "the lacteal secretion, practically free of colostrum, obtained by the complete milking of one or more healthy cows, which contains not less than 8.25% milk-solids-not-fat and not less than 3.25% fat." The term *milk* is understood as referring to cow's milk unless other species are mentioned specifically. For most of the world, particularly the West, milk from cattle accounts for nearly all the milk processed for human consumption [1]. However, other milking animals are very important to some populations because their milk provides an excellent and cheap source of highly valuable animal protein and other constituents. For example, sheep followed by goat make a major contribution to the milk production of the Mediterranean countries and also in large areas of Africa and Asia. Worldwide, the dairy industry produces milk as a fluid product and transformed into a variety of manufactured dairy products using a range of advanced processing technologies. The family of dairy products manufactured from milk is shown in Figure 11.1 [2].

11.2 COMPOSITION AND STRUCTURE

Milk is a polyphasic normal secretion of the mammary glands. Milk consists of (i) an oil-in-water emulsion with the fat in the form of droplets or globules dispersed in the continuous milk serum, known as whey; (ii) a colloidal suspension of proteins of various sizes in milk serum, consisting mostly of casein micelles, globular proteins, and lipoprotein particles; and (iii) a solution of lactose, soluble proteins, minerals, vitamins, and other components [3]. In addition, milk is a very complex food with over 100,000 different molecular species found, but most have not been identified [4]. The main component of milk is water. The remaining compounds are mainly fat (3.9%), protein (3.3%), lactose (5%), and minerals (0.7%) [5]. Milk also contains vitamins (e.g., vitamins A and C), enzymes (e.g., lactoperoxidases and acid phosphatase), and somatic cells [4]. The average composition of milk with respect to the major classes of compounds and a range of average values for milks of western breeds are shown in Table 11.1. There can be considerable compositional differences between species and even between breeds of a single species. The lipid content is the most variable fraction. Lipid is present mainly in the form of triglyceride, which makes up about 98% of milk fat. The remaining 2% consists of diglycerides, monoglycerides, cholesterol, phospholipids, free fatty acids, cerebrosides, and gangliosides [4]. The major fatty acids of milk are C_{14}, C_{16}, C_{18}, and $C_{18:1}$ fatty acids [6]. The fat is present in fresh milk mainly in the form of fat globules surrounded by a phospholipid-rich layer known as the milk fat globule membrane. Milk proteins are fractionated in two main groups: the casein fraction and the whey proteins. Caseins precipitate out of solution upon acidification of milk to pH 4.6 at 20°C, while whey proteins remain soluble under these conditions. Caseins can be fractionated into four main proteins: α_{s1}-, α_{s2}-, β-, and K-caseins [4]. Whey proteins include mainly β-lactoglobulin, α-lactalbumin, serum albumin, lactotransferrin, immunoglobulins, and β_2-microglobulin [6]. Lactose is the predominant sugar in milk; other carbohydrates are present in trace amounts and are mainly galactose and glucose. The most important function of lactose is as a fermentation substrate for lactic acid bacteria.

11.3 QUALITY CRITERIA FOR MILK

Milk is an important raw material for the production of a variety of dairy products. It is therefore important that the

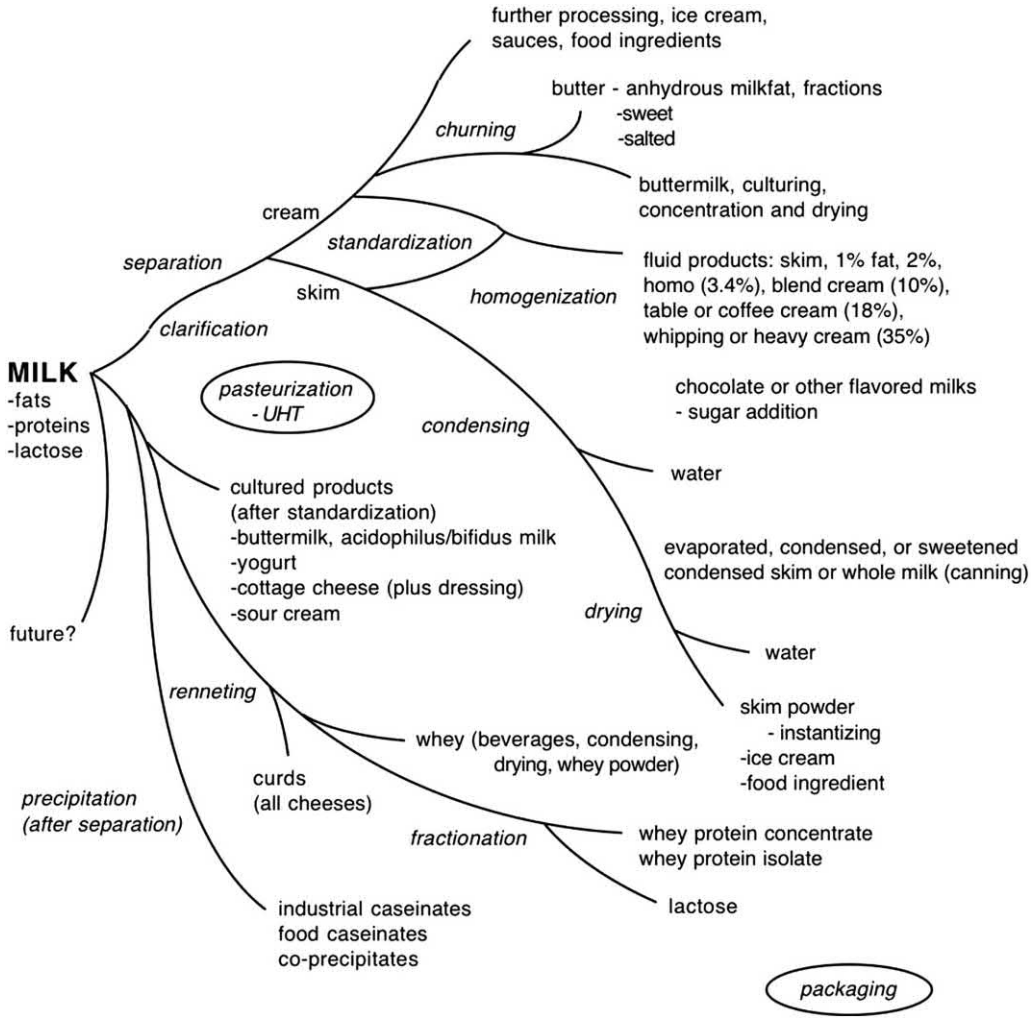

FIGURE 11.1 Family of dairy products manufactured from milk. (From Goff and Sahagian [2].)

milk used for processing has acceptable quality characteristics. Quality characteristics for raw milk include compositional quality, microbial contamination levels, somatic cell count, freedom from inhibitory substances and reception temperature [7].

The most common grades of raw milk in the United States are Grade A and Manufacturing Grade. The dairy farmer must meet state and federal standards to produce Grade A milk. In addition to the state requirements, a few municipal governments also have raw milk regulations. The dairy farmer must have healthy cows, have adequate facilities (barn, milk house, and equipment), and must maintain satisfactory sanitation of these facilities. The Food and Drug Administration's Pasteurized Milk Ordinance (PMO 2001) [8] requires that Grade A milk not exceed 100,000 CFU/ml standard plate count (SPC) for an individual milk producer, 300,000 CFU/ml SPC as commingled milk, and 750,000 cells/ml somatic cell count (SCC). In addition, good quality milk must not contain pesticides, antibiotics, sanitizers, drug residues, and other abnormalities. The storage temperature should not exceed 7°C within 2 hours of milking. It is also important that the milk used for processing has acceptable flavor characteristics. Various weed, feed, and cowy flavors can be transmitted to milk by the cow's respiratory or digestive system. These are considered normal and acceptable up to certain level, although excessive amounts can cause off-flavors that are difficult to remove by processing. A salty flavor can arise from cows in late lactation and infected with mastitis. Milk diluted with water can taste flat and be lacking in typical flavor [9].

11.4 MICROFLORA OF RAW MILK

Milk is an excellent medium for the growth of a variety of microorganisms owing to its high water content, neutral pH (6.4–6.6), and ample supply of nutrients. Aseptically collected milk from clean, healthy cows typically has an SPC less than 1000. Higher SPCs indicate that milk was subject to contamination. Microbial contamination generally occurs from three main sources: from within the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment [10]. The contribution of some sources of contamination on the colony count of raw milk is shown in Table 11.2. Bacterial contamination from within the udder is frequently a result of mastitis, an inflammatory disease

TABLE 11.1
Composition of Milk from Different Sources

Component	Average Percentage (%)	Range for Western Breeds* (Average percentage, %)
Total solids	13.23	12.16–14.42
Fat	4.22	3.54–5.13
Protein	3.58	3.29–3.98
Lactose	4.78	4.68–4.94
Ash	0.74	0.72–0.77

Source: Webb et al. [43]

* Western breeds include Holstein, Brown Swiss, Ayrshire, Guernsey, Jersey, and Shorthorn.

TABLE 11.2
Contribution of Some Sources of Contamination on the Colony Count of Raw Milk[1]

Source of Contamination	Estimate of the Contribution to the Count (ml^{-1})
Udder of a healthy cow	Up to several thousand
Water for cleaning, rinsing	Up to several thousand
Udder of a mastitic cow	Up to several million
Dirty cows	A hundred up to several thousand
Dirty equipment	A thousand up to several million

Sources: Tetra Pak Processing Systems AB [32]; Murphy and Boor [44].

[1] Approximate examples.

of the mammary tissue. Many microorganisms can cause mastitis, the most important being *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Pseudomonas aeruginosa*, and *Corynebacterium pyogenes*. The first three of these are all potential human pathogens [11]. Sources of contamination from the exterior of the udder include water, soil, vegetation, and bedding material. In general, contamination with psychrotrophic bacteria has been associated with bedding material, untreated water, soil, and vegetation; coliform contamination with soil; and spore formers with bedding material [12, 13]. Therefore, milk is susceptible to contamination by two types of microorganisms: the pathogenic bacteria and the spoilage bacteria. The presence of pathogenic microorganisms in milk may result in infection and threat to the consumer's health. The growth of the spoilage bacteria is more determinant to the shelf life of milk than that of the pathogenic flora. The spoilage bacteria degrade the milk through the production of enzymes. Four types of enzyme activity are encountered [14]: (i) lactose may be fermented to lactic acid resulting in a soured product; (ii) lipids are hydrolyzed by lipase—both microbial and the native milk enzyme—and, as a result, rancidity develops; (iii) proteinase activity results in breakdown of milk proteins with both physical and organoleptic effects, principally gelation and the development of intense bitter flavors; and (iv) phospholipases can attack the milk fat globule membrane, which stabilizes the native emulsion of milk fat.

Raw milk contains a diverse and complex microbial population [15, 16]. Raw milk contains a high number of lactic acid bacteria (LAB) populations that include *Lactococcus* (8.2×10^1 to 1.4×10^4 CFU ml^{-1}), *Lactobacillus* (1.41×10^1 to 1.5×10^4 CFU ml^{-1}), *Leconostoc* (9.8×10^1 to 2.5×10^3 CFU ml^{-1}), and *Enterococcus* spp. (2.57×10^1 to 1.58×10^4 CFU ml^{-1}). Other microorganisms are also present in significant proportions; these include psychrotrophs such as *Pseudomonas*, *Acinetobacter*, and *Aeromonas* spp., which flourish during cold storage [17]. Milk contains a more diverse microflora than that originally reported owing to new developments in DNA sequencing technologies. A recent study using high-throughput DNA sequencing detected 256 bacterial species in raw cow's milk that was to be used for cheese production [18]. Among these bacteria, *Streptococcus thermophilus* and *Lactococcus lactis* dominated in the milk, representing 43.7% and 19% of the total, respectively. Other microorganisms previously associated with raw milk have also been detected. These include *Acinetobacter*, *Aeromonas*, *Brevibacterium*, *Corynebacterium*, *Lactobacillus*, *Pseudoalteromonas*, *Pseudomonas*, and *Staphylococcus*, representing between 1.3% and 3.7% of the total [18].

Once milk leaves the cow, the retention or preservation of milk quality requires cleanliness, sanitation, and careful handling. Undesirable changes in raw milk are initiated by microbiological growth and metabolism or by chemical or enzymatic reactions. Temperature is critical for dairy food quality and shelf life. Cold temperatures are used to minimize microbial growth in raw milk until it can be processed and to extend the shelf life of nonsterile dairy foods. A reduction in temperature below the minimum necessary for microbial growth extends the generation time of microorganisms and in effect prevents or retards reproduction. This is clearly shown in Figure 11.2, which illustrates the likely effect of

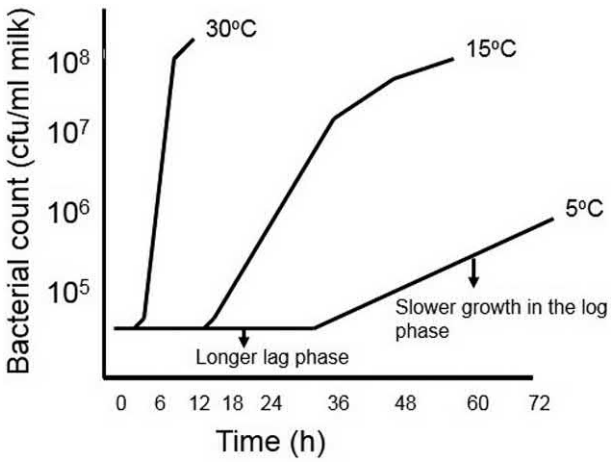

FIGURE 11.2 The effect of storage temperature on the bacterial count of raw milk having an initial SPC of 50,000 CFU/ml. (From Singh and Bennett [4].)

temperature on milk having an initial SPC of 50,000 CFU/ml. The microorganisms in raw milk just prior to pasteurization may include heat-susceptible pathogens as well as spoilage types [9]. Psychrotrophs became an escalating problem for the dairy industry during the introduction of refrigerated storage of raw milk. Psychrotrophs are of primary concern to the dairy industry since they can grow and cause spoilage in raw and processed dairy products commonly held under refrigeration. Psychrotrophic microorganisms capable of growing in milk at temperatures close to 0°C are represented by both Gram-negative and Gram-positive bacteria. For example, the gram-negative bacteria are *Pseudomonas*, *Achromobacter*, *Serratia*, *Alcaligenes*, *Chromobacterium*, and *Flavobacterium*; and gram-positive bacteria are *Bacillus*, *Clostridium*, *Corynebacterium*, *Streptococcus*, *Lactobacillus*, and *Microbacterium* [19, 20]. In aerated milk at 4°C, many strains of *Pseudomonas* spp. can produce sufficient proteinases to hydrolyze all of the available casein into soluble peptides [21, 22]. The enzyme activity from psychrotrophs stimulates the growth of starter lactic acid bacteria in milk [19]. Most psychrotrophs normally would not be a serious problem in milk because they are eliminated by pasteurization or ultrahigh temperature (UHT) treatment. However, psychrotrophs produce thermostable proteolytic enzymes, most of which attack κ-CN, resulting in a destabilization of the casein micelles and coagulation of the milk in a manner that is analogous to chymosin [23]. The quality of milk may be affected by heat-resistant enzymes secreted by psychrotrophs in raw milk before heat treatment, or other enzymes and metabolites that are produced by microflora during cold storage. Some of these enzymes are not inactivated by pasteurization or by other heat treatments and these may degrade milk products, even when the bacterium is destroyed. The shelf life of various dairy products is given in Table 11.3.

The spoilage of milk and dairy products is characterized by taste and odor changes, such as sour, putrid, bitter, malty, fruity, rancid, and unclean. The type of spoilage may also cause undesirable body, texture, and functional changes [9]. In milk about 40% of the milk solids are lactose, a major substrate for microbial fermentation in milk. Microorganisms use one of the two following methods to start fermentation: by the lactase enzyme (β-D-galactosidase) or by hydrolyzing the phosphorylated lactose by β-D-phosphogalactoside galactohydrolase. Microorganisms containing the lactase enzyme include *E. coli*, *S. thermophilus*, *L. lactis*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, and *Bacillus subtilis*. Lactic acid bacteria convert lactose to lactic acid and other by-products. Milk with a detectable acid/sour flavor is considered unacceptable commercially. Cold storage temperatures and sanitary storage and processing conditions for raw milk and cream can prevent the development of high acid/sour flavors [9].

A malty flavor or odor can occur in milk if *Streptococcus lactis* var. *maltigenes* grows and metabolizes amino acids in milk to aldehyde and alcohols. The fruity flavors in dairy foods can be caused by the metabolic activity of lactic acid and psychrotrophic bacteria with the formation of esters. Flavor defects in milk described as putrid, bitter, and unclean may be caused by the growth and metabolism of psychrotrophic bacteria. The lipase enzyme is often active at low temperatures, causing a lipolyzed flavor. Gram-negative psychrotrophic bacteria have lipolytic activity [9]. Table 11.4 summarizes the most important types of spoilage and the microorganisms responsible.

11.5 CONTROL OF MICROORGANISMS IN RAW MILK

Raw milk has been associated with several pathogenic bacteria such as *Campylobacter jejuni*, Shiga toxin-producing

TABLE 11.3
Shelf Life of Dairy Products

Product	Temperature (°C)	Shelf Life (Days)
Marketed milk	<4	12–14
Cottage cheese	2–4	15–30
Yogurt, sour cream, and dairy dip	<4	30–60
Curd cheese	<4	Several months

Source: LaGrange and Hammond [9].

TABLE 11.4
Some Types of Spoilage of Milk

Type of Spoilage	Microflora
Souring	Lactic acid bacteria
Casein precipitation	Lactic acid bacteria producing enough acid to drop the pH below 4.6
Gas production	*Clostridium*, *Bacillus*, yeasts, coliform bacteria, heterofermentative lactics, and propionics
Proteolysis	*Psychrotrophic bacteria: Streptococcus faecalis* var. *liquefaciens, Bacillus cereus, Micrococcus, Pseudomonas, Flavobacterium, Acinetobacter, Aeromonas* Thermophilic organisms: *Streptococcus* and *Lactobacillus* Spore-forming organisms: *Bacillus*
Lipolysis	Psychrotrophs: *Pseudomonas* spp., *Achromobacter* spp., *Alcaligenes* spp., *Acinetobacter* spp. Thermoduric organisms: *Streptococcus* and *Lactobacillus* Spore-forming bacteria: *Bacillus*
Ropiness	*Alcaligenes viscolactics*, *Enterobacter*, lactics
Changes in butterfat	*Pseudomonas*, *Proteus*, *Alcaligenes*, *Bacillus*, *Micrococcus*
Numerous off-flavors	*Pseudomonas*, *Actinomyces*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter Proteus*, *Lactococcus lactis* var. matigenes, molds, yeasts, coliforms, and mastitis-causing organisms
Color changes	*Pseudomonas syncyanera*, *Pseudomonas synxantha*, *Serratia marcescens*, *Pseudomonas fluorescens*

Sources: Banwart [45]; Fields [46]; Hayes and Boor [47].

E. coli, *L. monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* [15], and the risk of infection increases if milk is consumed raw [16]. Outbreaks linked to the consumption of cow's milk and cheese were estimated to cause on average 761 illnesses and 22 hospitalizations per year in the United States, mostly from *Salmonella* spp. and *Campylobacter* spp. Unpasteurized products are consumed by a small percentage of US dairy consumers but cause 95% of illnesses; the risk for illness was found to be 840 times higher for consumers of unpasteurized milk or cheese than for consumers of pasteurized dairy products [24].

Microbial growth and contamination can be prevented, slowed, or reduced by many means: (i) cleaning and sanitizing of the milk-handling equipment and the environment, (ii) holding the milk at low temperature, (iii) use of antimicrobial systems, (iv) thermization, and (v) clarification.

11.5.1 Cleaning and Sanitizing

Hygienic processing of food requires that the equipment is cleaned frequently and thoroughly to restore the equipment to the desired degree of cleanliness. The degree of cleanliness of the milking system probably influences the total bulk milk bacterial count as much, if not more, than any other factor [25]. Since proper cleaning and sanitizing of dairy equipment are important for the production of milk with acceptable microbial quality, control of psychrotrophs should begin at the farm level [12]. Psychrotrophic bacteria tend to be present in higher counts in milk and are often associated with occasional neglect of proper cleaning and sanitizing procedures [25]. Cleaning of dairy facilities involves removing soil from all surfaces that come into contact with milk and using a sanitizer after each processing period. Soil in the dairy industry is mainly minerals, lipids, carbohydrates, proteins, and water. Soil may also contain dust, lubricants, microorganisms, cleaning compounds, and sanitizers [26]. Microbial cleaning, also known as sanitizing or disinfection, is used to reduce the load of microbial contaminants that may be present on milk contact surfaces. Most chemical sanitizers used in the dairy industry kill off a broad spectrum of microorganisms, provided that they are used properly. Sanitizers commonly used in the dairy industry include chlorine compounds, iodophors, quaternary ammonium compounds (QUATs), acid anionic surfactants, and peroxyacetic acid. All disinfectants are deactivated to some extent by organic matter. This is why they are best used after thorough cleaning has removed most of the soil [11]. Many dairy plants use hot water as a common method of sanitation. This can be achieved by circulating water of 76°C to 85°C for at least 5 minutes, followed by a cooling chemical sanitizer rinse. Hot-water sanitation requires careful control to ensure that the required temperature is maintained long enough for it to be effective. This can be achieved by the use of thermostat-controlled tanks, which will circulate the water and maintain the desired temperature [27–29]. Hot water will often provide greater kill and longer milk shelf life than can be achieved with chemical sanitizer alone.

11.5.2 Cooling of Milk

Milk leaves the udder at a temperature of about 37°C, which is favorable for the growth of a large number of microorganisms, mainly mesophiles. Milk should therefore be quickly cooled on the farm after leaving the udder and stored in refrigerated bulk tanks at <7°C prior to collection. Collection by an insulated tanker is often done on alternate days and therefore some of the milk in the tank could be 48 hours old at the time of collection. Temperature control at the time of collection is thus critical to minimize microbial growth. Bacterial numbers in the milk may increase during transport as a result of contamination from surfaces of inadequately cleaned tankers or from the growth of psychrotrophic bacteria. Milk temperature and duration of transport are therefore important factors. On arrival at the processing site, the milk is usually transferred to bulk storage tanks prior to processing and cooled rapidly. Cooling is the main means of slowing the growth of bacteria in milk. The maximum storage time of milk is closely related to the storage temperature. Milk is typically stored at refrigeration temperatures that reduce the growth of most bacteria, with the exception of psychrotolerant microorganisms that can proliferate under these conditions and become a major cause of milk spoilage [30, 31]. This is primarily a result of the production of extracellular enzymes, mainly lipases and proteases. These lipases degrade milk fat causing rancidity, while proteases degrade casein producing a gray color and bitter off-flavors [31].

Low-temperature storage can reduce the frequency of raw milk collection from dairy farms to just two or three times a week, and enable further storage of milk in the dairy plant over weekends [19]. Spray and immersion coolers are commonly used on farms, which deliver milk to the dairy in tanks. In spray cooling, circulating chilled water is sprayed onto the outsides of the cans to keep the milk cool. The immersion cooler consists of a coil, which is lowered into the tank. Chilled water is circulated through the coil to keep the milk at the required temperature [32].

Where milking machines are available, bulk milk tanks, usually ranging from 0.8 to 19 m^3, are used to receive, cool, and hold the milk. As the cows are mechanically milked, the milk flows through sanitary pipelines to an insulated stainless steel bulk tank. An electric agitator stirs the milk, and mechanical refrigeration begins to cool it even during milking, from 32.2°C to 10°C within the first hour, and from 10°C to 4.4°C within the next hour. Some large dairy farms and collecting centers may use a plate or tubular heat exchanger to rapidly cool the milk. In these cases, the tank is mainly to maintain the required storage temperature. The temperature of the blended milk must be below 7.2°C during the second and subsequent milkings [33].

Since the milk is picked up from the farm tank daily or every other day, cooled milk may be stored in an insulated silo tank. Milk in the farm tank is pumped into a stainless steel tank on a truck for delivery to the plant or receiving station. The tanks are well insulated and the temperature rise should not be more than 1.1°C in 18 hours when testing the

tank full of water and the average gradient between the water and the atmosphere surrounding the tank is 16.7°C [33].

Most dairy processing plants either receive raw milk in bulk from a producer or arrange for pick up directly from the dairy farms. Storage tanks, from 4 to 230 m^3 made of stainless steel lining and well insulated, may be required for nonprocessing days and emergencies. The average 18-hour temperature change should be no more than 1.6°C in the tank filled with water, and the gradient to the surrounding air 16.7°C. For horizontal storage tanks, the allowable temperature change under the same conditions is 1.1°C. The tank may need cooling depending on the initial milk temperature and holding time. A plate heat exchanger may be connected or the tank surface, around the lining, may be cooled by passing a refrigerant or by circulation of chilled water or glycol solution. Agitation is essential to maintain uniform milk fat distribution. Milk held in large tanks, such as the silo type, is continuously agitated with a slow-speed propeller driven by a gearhead electric motor or with filtered compressed air [33].

11.5.3 Antimicrobial Constituents

11.5.3.1 The Lactoperoxidase System

There are some naturally occurring antimicrobial systems present in raw milk that might improve its shelf life. The main representative of these systems is lactoperoxidase. The milk enzyme lactoperoxidase catalyzes the oxidation of thiocyanate by hydrogen peroxide to produce antimicrobial substances. The inhibitory substances are claimed to be short-lived intermediary compounds, such as hypothiocyanate, cyanosulfurous acid, and cyanosulfuric acid [34]. Hypothiocyanate can kill gram-negative bacteria and inhibit gram positives, possibly by damaging the bacterial cytoplasmic membrane [11].

The lactoperoxidase system (LP) consists of three components: lactoperoxidase, thiocyanate, and hydrogen peroxide. All three components are required for antimicrobial activity. The enzyme is available in milk in abundance, however, the availability of thiocyanate in milk for the proper LP preservation is not sufficient. Certain bacteria in milk produce small quantities of hydrogen peroxide, but the quantity of oxygen that can be provided is too small for the oxidation process in the LP system. Stimulation of lactoperoxidase activity through the addition of exogenous thiocyanate and hydrogen peroxide has been investigated as a means of preserving raw milk in developing countries where ambient temperatures are high and refrigeration is not often available. For proper LP preservation, very small quantities of thiocyanate (0.00015%) and hydrogen peroxide (0.00085%) must be added to milk [35]. These quantities are sufficient to preserve milk at tropical temperatures for about 8 hours, whilst the preserved milk can be easily kept overnight at temperatures of 15°C to 20°C; at temperatures of 4°C, the milk can be kept for a few days without spoilage. Similarly, Bjorck et al. [36] studied the effect of this system on the quality of raw milk in developing countries. Their results showed that the quality of treated milk was significantly improved over that of the untreated control. Furthermore, they demonstrated that the length of bacteriostasis is temperature-dependent: 7 to 8 h at 30°C, 11 to 12 h at 25°C, 15 to 16 h at 20°C, and 24 to 26 h at 15°C. The International Dairy Federation (IDF) [37] recommended the addition of hydrogen peroxide and thiocyanate at about concentrations of 10 to 15 ppm in order to activate the LP system and extend the shelf life of raw milk.

11.5.3.2 Hydrogen Peroxide

Hydrogen peroxide is a preservative that has been used for a long time to preserve raw milk, under conditions where it may be difficult to cool the milk quickly. The concentrations required (300–800 ppm) are much higher than those required to activate the lactoperoxidase system [38]. For milk of reasonably good quality, 0.03% to 0.05% of pure hydrogen peroxide may be used to extend the keeping quality by at least 5 hours, depending on a number of conditions, such as temperature, catalase content of the milk, presence of heavy metals, and type of contaminating microorganisms [35]. In one trial in Africa, the addition of hydrogen peroxide increased the proportion of samples passing the 10-minute resazurin quality test from 26% to 88% [11]. Treatment levels of 0.115% completely inactivated *Mycobacterium tuberculosis*. Hydrogen peroxide is more effective at increased temperatures. A level of 0.8% by weight combined with a temperature of 49°C to 55°C for 30 minutes has been suggested as a substitute for pasteurization [38]. Anaerobic and coliform bacteria are more resistant than lactic acid and anaerobic bacteria [35]. Gram-positive bacteria are not inactivated by hydrogen peroxide to the same extent as gram-negative bacteria [39].

11.5.4 Thermization (Thermalization)

Often, a dairy is unable to process all milk supplies within 4 days of milking. Consequently, measures must be taken to keep the raw milk for a longer time. Dairy processors in European countries use a process called thermization to prevent psychrotrophs from growing in milk [28, 40]. Thermization is a mild thermal process applied to milk that may need to be stored over a long period prior to use. Thermization has now been defined as a heat treatment that uses temperatures between 57°C and 68°C for 15 seconds [38]. The purpose of this treatment is to protect against microorganisms that may grow during the storage of raw milk, especially gram-negative psychrotrophic bacteria. These bacteria produce heat-resistant lipases and proteinases that may eventually cause deterioration of milk products [41]. Thermization should be applied as soon after milk treatment as possible and it is only effective if thermized milk is kept cool (4°C) [38]. Thermization is a far better method of controlling the quality of dairy products than merely cooling the raw milk, but it also is more expensive. Except for the killing of many vegetative microorganisms, thermization causes almost no irreversible changes in milk [41]. However, some problems associated with thermization were reported by Muir [14]. One problem is associated with the contamination of thermized milk with gram-positive cocci such as *S. thermophilus* as a result of a buildup in the regeneration section of a commercial thermization unit.

Thermization may also slightly affect the flavor and texture of cheese but not the yield.

11.5.5 Clarification

Clarification is a commonly employed pretreatment of milk prior to its storage/manufacture into other products. The shelf life of milk can be extended by clarification. Clarification may be as simple as filtration or may include high-speed centrifugation to remove microbial cells and spores. Filtration is usually carried out by pumping milk through specially woven cloth. This results in the removal of debris and all extraneous matter.

Bactofugation refers to a high-speed centrifugation process carried out in a specifically designed separator, called a clarifier. The purpose of bactofugation is to separate bacterial cells and spores. The process is particularly important in Europe where it has been used in the cheese industry to remove spores from cheese milk that could cause latent fermentation in some types of cheeses. Bactofugation has also been adapted to processing drinking milks, where it succeeds in prolonging the shelf life of fresh, pasteurized milk by 3–5 days as a result of a reduction in the microbial population [42]. In addition, a reduction in the microbial population induces a reduction in the pasteurization temperature and consequently the manufacture of a product with improved flavor.

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12 Quality Assessment Methods and Postharvest Handling of Fresh Poultry Eggs

Mohammad Aboonajmi and Hamideh Faridi

CONTENTS

12.1 INTRODUCTION

One of the main objectives of food processing is to achieve uniform quality of raw materials and the final products. The demand for reliable, fast, and non-destructive quality assessment methods and postharvest handling of poultry egg has resulted in the development of many principles. The quality of poultry eggs is evaluated based on the physical, chemical, biological, and functional properties of eggs. The quality of eggshell is largely influenced by storage conditions, such as temperature and relative humidity, and stress conditions, such as impacts and vibration caused during transport and handling. In this chapter, the postharvest handling of eggs and quality assessment methods are described.

12.2 QUALITY ASSESSMENT METHODS FOR FRESH POULTRY EGGS

12.2.1 Overview

The quality of egg and the healthy conditions of layer flock can be carried out to correct problems before they create large economic losses or hygienic problems. The contaminations of eggs with bacteria like *Salmonellae* are common. The presence of a fragile eggshell requires a sophisticated gentle way of handling in the automated grading systems. Quality consists of the interior and external characteristics of eggs. Most of the exterior quality aspects are related to the eggshell and include shell integrity, color, thickness, porosity, and strength. Other external quality aspects are the presence of dirt on the shell and cuticles. Except for the shell color, these external quality characteristics are related to the protection of interior contents by avoiding physical damage and bacterial invasions.

In general, internal egg quality relates to the characteristics of the albumen. The albumen quality decreases with the storage time due to biochemical reactions, which are governed by complex internal and external factors, such as temperature and relative humidity as well as the presence of bacteria. The albumen transforms into a watery substance from a highly viscous component over several weeks. Therefore, albumen quality is often taken as a yardstick to evaluate albumen (i.e. egg) freshness. Historically, albumen quality is estimated by Haugh units [1], which measure the albumen height after breakage and are empirically corrected by egg mass. This technique has some important disadvantages although it is an easy method. Albumen can include dissolved blood, bloodspots, and meat spots (i.e. inclusions other than blood).

Ovomucin, one of the major egg white proteins, has wide potential applications in the food, functional food, and nutraceutical industries due to its unique physicochemical properties and bioactivities [2–7]. Although ovomucin can be easily precipitated from diluted egg white at acidified pH, the method for preparing high-purity ovomucin is challenging due to the co-precipitation of lysozyme and ovalbumin [8–10]. In literature, the purity of ovomucin prepared by the precipitation

method was less than 70%. For research purposes, pure ovomucin can be prepared by the gel filtration method; however, this method is not suitable for large-scale production [11]. A new two-step method was recently reported that was able to prepare ovomucin with over 90% purity [12]. The storage of eggs is known to lead to quality deterioration or egg white thinning [13]. The storage of eggs was also reported to affect the physicochemical properties of egg white [14]. Schafer et al. [15] reported that storage did not affect the content of lysozyme and ovotransferrin, while ovalbumin decreased after 6 weeks of storage. Since shell eggs inevitably have to be stored before processing, it is not known if storage of the egg might affect the extractability of ovomucin.

A new two-step method was recently developed to prepare high-purity ovomucin by Wang et al. [16]. In this study, the effect of shell egg storage on ovomucin extraction was investigated. The composition of ovomucin extracts from egg white was determined by gel filtration chromatography. Both storage temperature and time could significantly ($p < 0.05$) affect the purity and yield of the ovomucin. After nine weeks of storage at 4°C, the content of the ovomucin in the extract decreased from 92.5 to 82.4%, and the yield of ovomucin decreased from 214 to 120 mg/100 g of egg albumen (egg white). After 5 weeks of storage at 22°C, the content of ovomucin extract decreased from 92.5 to 73.0%, and the yield of ovomucin decreased from 214 to 112 mg/100 g of egg albumen. The increase in egg white pH during prolonged storage leading to degradation of ovomucin is very likely responsible for the decreased extractability of ovomucin.

The rate at which eggs are graded today is up to 180,000 eggs/hour; thus this task cannot be controlled individually by a trained human eye. Therefore, the egg-grading equipment is equipped with specially developed sensor devices to estimate one or more quality aspects. The devices need to be non-destructive and fast. These novel devices are commonly compared to the classic tests for quality assessment, which are usually destructive and time-consuming.

Visual inspection is commonly performed to evaluate the quality of egg. However, the required grading rate makes visual inspection impractical since huge numbers of eggs need to be assessed within a very short time. Therefore, the development of new non-invasive and non-destructive apparatus to measure the quality of eggs is needed. Three main types of quality-sensing devices are commercially available. The first type is based on mechanical techniques, which measure eggshell properties, such as the presence of cracks and the eggshell strength. Spectroscopic principles are the basis of the second type of sensors used to assess the interior egg quality. At the beginning, the spectroscopic details were used on the basis of one or a limited number of wavelengths (e.g. to discover blood or other inclusions). Later, advanced spectroscopic facilities used hyperspectral data to determine albumen properties like Haugh units, pH, and viscosity. Finally, computer vision techniques are used to acquire information on the external quality of eggs (e.g. eggshell color and dirty shell detection). They can detect quickly the cracks which could be a major problem in the hygiene and cleanliness of the egg. Recently, researchers are paying attention to modern sensors, which are based on ultrasonic, magnetic resonance and electronic nose techniques. In addition, the combination of two different measurement methods (e.g. eggshell conductance) could be assembled.

12.2.2 Mechanical Techniques

A lot of mechanical techniques have been used to evaluate the physical qualities, like the strength and the integrity, of the shell. A whole set of mostly destructive and time-consuming protocols is reviewed by Hamilton [17]. These techniques are often used as reference techniques to evaluate the development of fast and non-invasive techniques. For the non-invasive evaluation of eggshell strength and integrity, a mechanical technique is used to detect hair cracks in commercial egg-grading machines. In these devices, the local integrity of an eggshell is assessed by measuring the number of rebounds of a small impactor on it.

Several elastic rebounds of this impactor denoted a locally intact eggshell. Close to a crack in the eggshell, the elasticity of the eggshell impairs the rebounds of the impactor (i.e. heavily damped). This is reflected in a serious reduction of the rebounds number of the impactor. Utilizing these measurements on several locations, cracks all over the egg can be discovered. In one case, a small ball moving an electromagnetic probe has the roll of an impactor [18]. In another commercially available application, the impactor is the egg itself and the rebounds are counted by a piezoelectric element [19]. In these methods, the crack detection rate varies from 70 to 85%.

Coucke [20] and later De Ketelaere et al. [21] evaluated the dynamic response of eggs after an excitation. After excitation, the egg exhibits a complex damped harmonic vibration. For an unbroken eggshell, this vibration is identical wherever measured on the equator of the eggshell. Therefore, it is possible to judge the integrity of the eggshell by comparing the frequency spectrum measured at various places on the equator of the vibrating egg. Broken shells show a different response at different places on the equator. Up to 90% of the cracked eggs are detected using this fast technique since it works at about 7 m/s. By applying discriminant analysis and a multiple regression technique, Cho et al. [22] increased the detection rate by up to 94%. Jindal and Sritham [23] detected almost 99% of the cracked eggs using neural networks to compare the recorded spectra. The possibility of detecting cracked eggshells by comparing the vibrational behavior at the blunt end and at the equator after excitation was shown by Wang and Jiang [24]. Further, the dynamic stiffness from the measured frequency spectrum was measured for intact eggshells [25, 26]. This dynamic stiffness was correlated with other parameters of the eggshell strength, such as static stiffness and eggshell thickness. An acoustic technique for measuring the dynamic stiffness could be used to predict the cracks in eggs during handling and transport [27]. Micro-organisms, such as *S. enteritidis*, can easily penetrate into eggs which have low dynamic stiffness [28].

The eggshell is fragile, and cracked eggs are more vulnerable to bacterial infections, which can lead to health hazards. Hence, detection and removal of cracked eggs continue to be very important for quality assurance in the production and marketing of eggs [29, 30]. There are two main methods prepared to detect eggshell cracks including vibration-based response analysis and machine vision inspection [30]. Many studies have shown that the vibration-based response analysis is a more effective detection method than the machine vision inspection method, especially for the detection of hairline cracks and invisible cracks [7, 31]. In the vibration-based detections, Fourier analysis is broadly used to find crack-sensitive indicators by comparison of the feature difference between cracked and intact eggs. For instance, De Ketelaere et al. [31] found that for intact eggs, the impulse responses are nearly identical at every point on the eggshell equator, whereas eggs with a damaged shell show different responses at different locations of the equator. A crack detection algorithm based on the correlations between the repeated measurements obtained from the same egg (four repetitions) was suggested by the authors [31]. The magnitudes of the peak frequencies (peak frequencies indicate the frequency of the highest peak, the frequency of the second peak, and so on) were found to be similar for the cracked eggs and showed that the first dominant resonance frequency values of the cracked eggs were lower than those of the intact eggs [24]. Jindal and Sritham [23] classified eggs using artificial neural network techniques based on the vibration after impact and reported 99% crack detection and 10% false rejection. The physical properties of eggshells were also analyzed using vibration-based methods. Coucke et al. [25] measured the mechanical stiffness of an eggshell through resonance frequency analysis and stated a correlation of 0.71 between the static and dynamic stiffness of an eggshell. Wang et al. [24] explained the relationship between the dynamic resonance frequency and the eggs' physical properties. They found that the dominant frequency rises while the shell stiffness or egg density increases, and decreases when the egg mass increases. Kemps et al. [32] investigated the incubation of eggs and its relation to embryonic development using the vibration analysis method. They found that the time at which the damping of the vibration suddenly changed, the diameter of the eggs, and their interaction were significant explanatory variables in predicting hatching time. Bamelis et al. [33] measured the conductance of eggshells using the acoustic resonance technique, based on the optical transmission spectra. The authors found that the dynamic stiffness of an egg and the optical transmission at 611 nm are the parameters with the highest predictive power when estimating eggshell conductance. It is also worthwhile to point out that vibration-based analysis techniques have been broadly applied in the quality detection and control of agricultural products [34–39].

It is well-known that cracks in table eggs increase the risk of bacterial contamination, and the removal of cracked eggs has been a USDA grade standard for many years [40, 41]. Cracks still frequently occur throughout various points of egg collection and processing, and there are numerous high-speed online commercial crack detectors in use. The accuracy of crack detectors is validated by USDA human graders to ensure that they are in compliance with voluntary grade standards (USDA, 2005). However, to validate the high-speed systems, human graders still rely on hand-candling techniques with somewhat antiquated candling lights. In recent years, especially at integrated egg processors with fresh eggs, USDA graders have been having difficulty in detecting small hairline cracks and micro-cracks [42]. Lawrence et al. [43–45] have developed and patented an imaging system to assist the graders in detecting these fresh cracks in table eggs. The system was designed for white table eggs and took about 60 seconds to process a set of 15 eggs. In a 1000-egg study with approximately 350 fresh hairline-cracked eggs, the system was 99.6% accurate with only a 0.2% false rejection rate (intact eggs incorrectly classified as cracked). The system had a higher accuracy than any other crack detector currently in use [29, 46]. The imaging-based system used a high-resolution digital camera to capture an image of 15 eggs in a transparent enclosure. Then a small, quick negative pressure was applied to the enclosure, which opens any eggshell cracks, while a second image was taken. The comparison of the two images was then used to detect any change in the image intensity, which was associated with a crack. Jones et al. [47] showed that the system did not affect egg quality, including Haugh units, albumen height, egg weight, shell strength, vitelline membrane strength and elasticity, and whole-egg total solids, during cold storage [47]. Recently, additional modifications to the system have been made, including enlarging the enclosure to hold 20 eggs, automating the rollers, adding a case light for external shell quality, and implementing a touch screen with data management software. Previous measurements on intact eggs had shown that the system did not cause cracks in intact eggs [44, 45]. However, in discussions with industry representatives, there was a concern about the system causing cracks in poor-shell-quality eggs. There are many factors that affect eggshell quality including adequate nutrition, flock health, management practices, environmental conditions, and breeding. However, it is well-known that consistently poor shell quality occurs in eggs laid from hens just prior to melt in the heat of the summer [48]. Lawrence et al. [49] reported the improvements to the system and the system's effect on poor-shell-quality eggs. To aid graders, a modified-pressure imaging system was developed to detect cracks in table eggs [49]. The original system was modified to grade 20 eggs at a time in batch mode. The eggs are positioned on rollers and held in a sealed clear acrylic chamber (Figure 12.1). The system utilizes high-intensity white LED lights for illumination, and a stepper motor is now used to automate the rotation of the eggs. It also uses a high-resolution monochromatic camera to take images of the eggs. The first image is taken at atmospheric pressure, and the second is taken while the chamber undergoes a short, rapid, negative change in pressure. If there is no crack, then there is effectively no difference in the two images. However, if a crack is present, it causes an increase in the intensity of the crack pixels in the negative-pressure image, resulting in a significant difference between the two

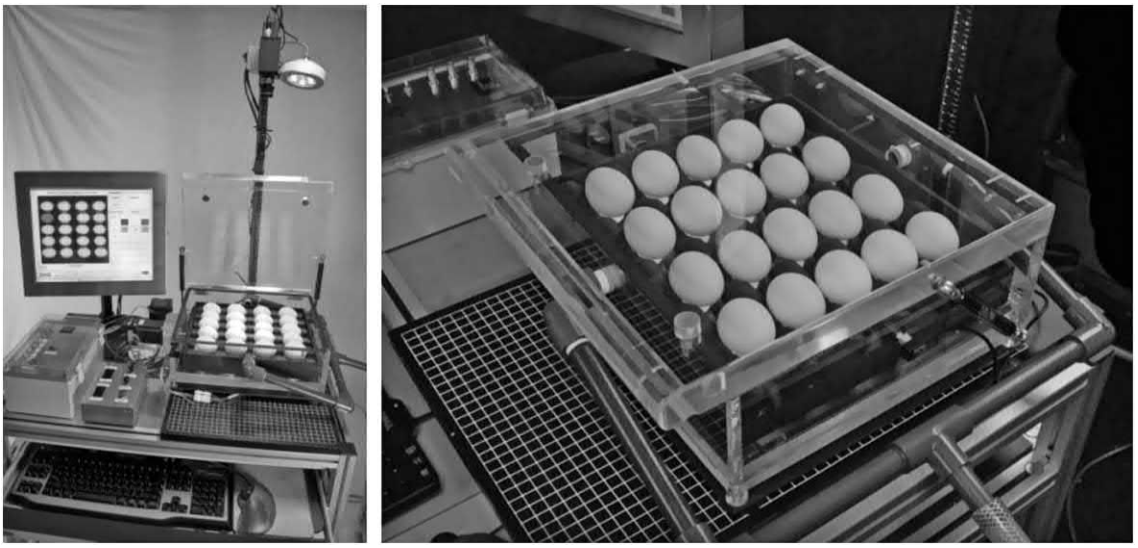

FIGURE 12.1 (Left) Crack detection system as shown: acrylic egg chamber with hinged lid, CCD camera, case light, control box, and touch-screen monitor. (Right) Close-up of the vacuum chamber holding 20 white table eggs. Note the two cylindrical magnets mounted in the lid to hold it closed. (From Lawrence et al. [49].)

images. This difference (in the form of a ratio) is the basis for identifying a crack. The software then displays the egg images and each egg is color-coded either green (intact) or red (cracked), and any cracks are highlighted with a contrasting color. Typically, the system uses four sets of images to cover practically all the eggs' surface and completes the crack detection in about 40 seconds. The modified system was tested with poor-shell-quality eggs from multiple strains of hens to see if it would induce cracks in these eggs. The analysis of 3279 poor-shell-quality eggs (both white and brown), from six strains of hens, laid in the heat of the summer, resulted in only one crack caused by the system (0.03%). Thus, it is highly unusual for the system to cause cracks in intact eggs, even eggs with poor shell quality.

The most useful information could be obtained from the inside of the egg, where the embryo is developing. Acoustic Resonance Analysis (ARA) has the ability to detect the embryo after 96 hours of incubation. By measuring the blood value retrieved from optical transmission spectra, embryonic presence is even found after about 72 hours of incubation [50]. Moreover, the dynamic stiffness can be determined by ARA to state the effect of heat stress on a layer flock [51] and to determine the risk of shell breakage at different stages of production.

Eggshell quality is important in selecting the program to choose new breeder flocks both for layers and broilers. The fast and non-invasive measurement of the dynamical stiffness might be a useful tool in breeding as it demonstrates a high heritability [50] and seems to be a good estimator for the risk indicator to determine cracks during handling and transport. Lin et al. [52] evaluated eggshell stiffness using an acoustic resonance system. The evaluation was achieved by analyzing the measured frequency response of eggshell excited with light mechanical equipment. Partial least squares (PLS), synergy interval PLS (si-PLS), genetic algorithm PLS (GA-PLS), and GA-siPLS algorithms were used to calibrate the regression model. The GA-PLS model can help to build a compact and robust model serving for on-line measurement of the stiffness of eggs.

For decades, breeding companies have used laboratory-based measurements, such as shell breaking strength, non-destructive deformation, and specific gravity [53]. While these measurements have generally helped in making a selection, it has been notoriously difficult to prove that they directly relate to the incidence of breakage based on the shell strength. This is due to the number of possible physiological responses to choose from for shell strength, including rate of shell deposition, uterine environment, changes in acid–base balance, and efficiency in metabolizing calcium [54, 55]. Eggshell strength is generally measured using either direct tests, such as non-destructive deformation [56, 57] or destructive fracture force [58–60] of an egg under quasi-static compression between two parallel plates, or indirect tests, such as the measurement of eggshell thickness [61–65] or specific gravity [66]. Many of these methods, however, are destructive, slow, or subject to environmental influences; hence these are regarded as impractical. Coucke [25] constructed a fast, objective, and non-destructive technology for the determination of eggshell strength based on acoustic resonance analysis. This method measured the egg resonant frequency (RF) and damping ratio. Based on the RF and egg weight, the dynamic shell stiffness (K_{dyn}) was defined. This technique can also be used to detect cracks in the eggshell [25, 26, 30, 67]. Many studies show the K_{dyn} to be a useful eggshell quality measurement. De Ketelaere et al. [31], for instance, investigated the variation of this strength parameter in relation to certain production parameters. A small-scale experiment also indicated a decline in K_{dyn} to be a result of heat stress [35]. Coucke et al. [30], De Ketelaere et al. [31], and Wang et al. [67] also found an acceptable correlation between the measurement of K_{dyn}

and other measures of eggshell quality. Eventually, K_{dyn} provided a good approximation of eggshell strength in relation to the likelihood of breakage in practice as shown by Bain et al. [32]. This created the opportunity to treat K_{dyn} as a new eggshell quality parameter in breeding programs. As most deformations that cause the eggshell to break in practice are dynamic, this might lead to an increased selection response as compared with the classic static techniques, but it needs to be proved. Researchers investigated the relationship between some measures of shell quality (specific gravity, breaking strength, shell thickness, percentage of shell, and shell weight per unit of surface area) and egg breakage in practice [68–71]. The mechanics and mechanisms of failure of eggs have been examined through punch tests, and these show the importance of establishing damage over a critical area before the macroscopic failure of the eggshell at the maximum load (i.e. one or more of the micro-cracks become unstable) [72]. Structural strength, on the other hand, is related to the interactions among the building units and depends on several variables, namely size, shape, thickness, and distribution of the shell components. Most techniques used to quantify eggshell strength measure the eggs as a whole and thereby make no distinction between properties of structural strength and mechanisms of failure of hens' eggs. For example, static stiffness (K_{stat}; 22) and dynamic stiffness (K_{dyn}; 5) are measured for total shell strength. The practical loads on the eggshell have a dynamic nature, and so the dynamic strength of an eggshell could be related better to conditions experienced during the handling and transportation of the eggs. Thus, there is apparently no ideal variable for the stability of eggshell strength. As a new non-destructive method for the stability of eggshell strength based on the different strains of laying hens with different ages in terms of their eggshell quality, the compression cone hardness was tested [73]. Seven measurements were used in the classical method, such as egg mass, shape index, static stiffness, breaking strength, shell thickness, and shell mass. Furthermore, the dynamic stiffness as mentioned by Coucke [25] was compared with the compression cone hardness. The results from multiple regressions revealed stronger influences on breaking force strength than static stiffness (deformation) due to the compression cone hardness and dynamic stiffness. It is necessary to develop other measurement methods to estimate eggshell quality without destroying the eggshell. Compression cone hardness (CCH), shell deformation, shell breakage strength, shell thickness, static stiffness, dynamic stiffness, and shell mass showed high coefficients of correlation. Intact eggs produced sound signals at a single dominant peak in the frequency range of 3000–8000 Hz with a duration of about 20 ms. The cracked eggs showed frequency spectra in a relatively wider frequency range of 2000–10,000 Hz and a shorter signal duration of about 15 ms. Finally, it was concluded that the influence of strength (i.e. breaking force) upon total eggshell strength (i.e. crack detector) is limited.

In the albumen, one of the most obvious changes is the physical deterioration of the thick white [74]. The gelatinous structure of the thick white gradually deteriorates to thin white during storage [75]. The "thinning" phenomenon was widely described by Raymond Haugh [76] as a good indicator of egg quality [77]. During storage, the vitelline membrane surrounding the yolk loses its structural integrity and stretches [78, 79]. The European Union legislation provides the measure of the air cell height for the commercial classification of eggs (EEC, 1991). The air cell height increases in volume for the loss of carbon dioxide with age. These changes are influenced by storage conditions, like relative humidity and temperature [80]. Wavelet analysis was introduced in the early 1990s to mathematically analyze signals from the time domain into different layers of frequency levels. The wavelet transform is described in a fashion similar to the short-time Fourier transform. It uses a waveform function called the wavelet instead of using periodic functions. Therefore, the wavelet transform is more suitable for the analysis of non-stationary signals by effectively extracting the time-frequency features of the signals; it has been developed as an alternative to Fourier transform in order to overcome problems related to the frequency resolution. More details of the wavelet function and its applications can be found elsewhere [81–87]. Fourier analysis is performed to find an effective crack feature for eggshell crack detection, and the wavelet analysis has not yet been utilized. For binary classification, a support vector machine (SVM) was initially designed, and its aim was to find a decision from the closed training points that has the maximum distance (margin) from the closest training points [88]. Many studies showed the SVM to be a powerful classification method in different applications [89]. Denga et al. [90] proposed a new detection methodology for eggshell cracks using a continuous wavelet transform and a support vector machine (SVM) technique (Figure 12.1). The proposed methodology included an experimental system and a data processing system, which was used to generate the impact force and to measure the acoustic signal, whereas the data processing system extracted timely signal features and classified the signals based on an SVM algorithm. This method led to achieving the highest crack detection rate of 98.9%, and the smallest false rejection rate of 0.8%.

Aboonajmi et al. [91] demonstrated the possibility of the non-destructive prediction of the main quality indices of commercial eggs by processing a short ultrasound burst passing through the egg material and calculating the ultrasound phase velocity. For this purpose, a set of 300 samples of commercial eggs (Boris Brown, 33 weeks of age) from the first day (i.e. egg lying) were purchased from a farm and classified in two groups. The first group was kept at room temperature (22–25°C) while the second group was kept in a refrigerator (4–5°C). Twenty-five eggs were picked every week from each group (room and refrigerator) and then subjected to the non-destructive ultrasound test at room temperature. Each day, the ultrasound signal was recorded from the eggs. Then the air cell, thick albumen heights, Haugh unit, and yolk index of the eggs were also determined by a destructive method for comparison. Both the Haugh unit and yolk index decreased with time over 5 weeks in storage at room and refrigerator temperatures, while the air cell height increased. The lower the Haugh unit for the eggs in the refrigerator, the lower the phase velocity (1573 m/s on the first day compared to 1540

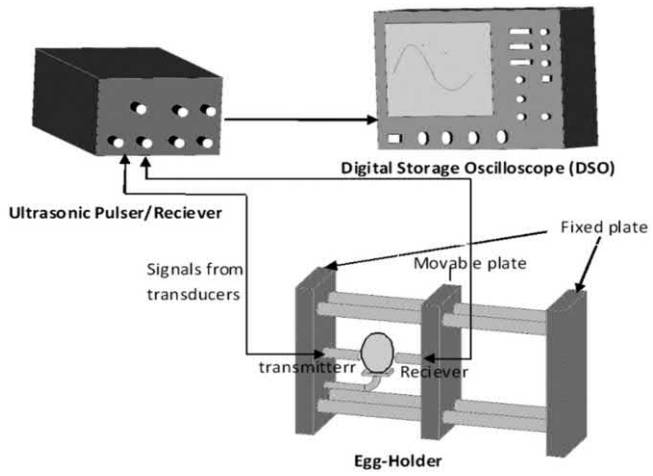

FIGURE 12.2 The experimental setup for ultrasonic testing of poultry eggs. (From Aboonajmi et al. [92].)

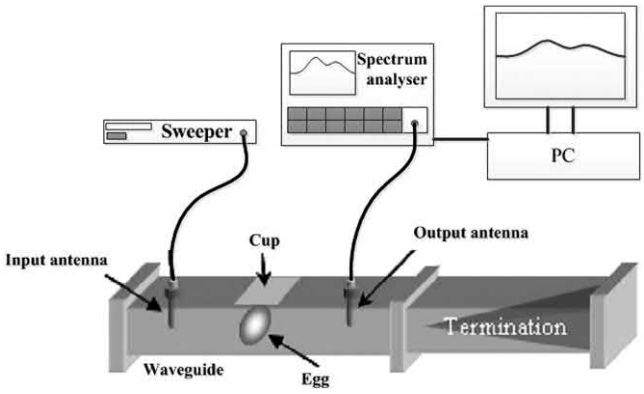

FIGURE 12.3 Layout of the used instrumental chain. (From Ragni et al. [93].)

m/s after 3 weeks). Similar changes of the phase velocity were found for the eggs at room temperature (1571 m/s on the first day compared to 1514 m/s after 3 weeks).

Another study was done by Aboonajmi et al. [92] using a non-destructive ultrasound method to develop a model for poultry egg freshness assessment (Figure 12.2). The proposed model can predict the Haugh unit, albumen thickness, air-cell height, and a number of other egg quality parameters by computing the ultrasound phase velocity within the egg material. For this purpose, the effect of the storage time on the ultrasound phase velocity within the poultry eggs and the peak values of the transmitted ultrasound signals in the time and frequency domains were considered to be indicators of egg freshness. Tests were conducted on eggs stored for 5 weeks in different storage conditions. The computed parameters were used to develop different models to predict the number of storage days for the egg samples. The results showed that the amplitude of the main peaks of the ultrasound signal in the time domain increased with the days of storage. Moreover, there was a significant difference between the mean values of the phase velocities obtained at different times during the storage period. Comparing the results obtained for the eggs kept at room temperature as compared to refrigerated showed that variations were more significant for the eggs kept at room temperature.

A waveguide technique for the non-destructive determination of egg quality parameters was carried out by Ragni et al. [93]. By applying a waveguide probe as an input for shell eggs to a sinewave sweeper oscillator, the signal at the output was captured by a spectrum analyzer (Figure 12.3). The correlations between the quality parameters and tests carried out on albumen, yolk, and plastic eggs for simulating the air cell showed how one index can be indirectly predicted through another one.

12.2.3 Spectroscopic Techniques

In general, light is composed of electromagnetic waves of a broad spectrum of wavelengths; the visible range (VIS) is within wavelengths between 300 and 750 nm and the near-infrared range (NIR) is between 700 and 2500 nm. This spectrum is changed due to physical and chemical interactions when it is passed through a material. Thus, the original spectrum can be compared with the changed spectrum, and optical information details of the object could be linked with the chemical and physical quality. Several authors have found reliable methods to link this optical information with the quality of eggs. The optical measurements have the advantage due to their non-destructive and fast measurement; hence the risk for cross-contamination can be avoided due to their non-contact with eggs.

Bamelis [94] demonstrated an inter-egg variation in optical transmission spectra on white-shelled eggs using visible light. The size of an egg and thickness of the eggshell varied during the storage time of eggs. From these optical spectra, the transmission ratio of two wavelengths, 674 and 663 nm, was calculated, and this ratio varied logarithmically towards a constant value; however, the time to reach this constant differed from egg to egg. The use of a time-dependent evolution of the optical spectra in the NIR range was also reported by Norris [95]. Kemps et al. [96] investigated the possibility of estimating albumen quality parameters, such as pH and Haugh units (HU), from the optically measured VIS/NIR transmission spectra. A correlation of 0.82 between predicted and measured parameters was reported. Schmilovitch et al. [97] predicted the pH from NIR transmission spectra as measured through whole eggs. Kemps et al. [98] reached the same results for pH and HU.

The optical spectrum can be influenced by optical active pigments and two pigment detection devices are available to detect hemoglobin and protoporphyrin. The blood and color of eggshell are measured by detecting their important pigments. First Brant et al. [98] suggested a technique to discover blood in a non-invasive way by selecting the absorption peaks of hemoglobin at 577 nm. Gielen et al. [99] showed that the range between 585 and 610 nm could be useful as a reference value to calculate the blood value. The presence of protoporphyrin in the shell of brown eggs was a big problem in the detection of blood, but protoporphyrin can be detected from the color of eggshells [100]. Besides protoporphyrin, biliverdin is an important eggshell pigment for the eggshell color [101]. Using the light reflected by an eggshell, Wei and Bitgood [102] developed a device to measure the eggshell color.

Another non-destructive assessment of the egg's internal quality and freshness is mainly performed by spectroscopic techniques, when the interaction of an electromagnetic wave with the product represents the common principle [103]. Similar studies have been carried out for other agricultural products during the last few years [94, 104–107]. The main results showed significant correlations of the visible near-infrared transmission spectra with the storage time [94] and the albumen quality in terms of Haugh unit, an expression relating to the thick albumen height and the egg mass [1]; for this last parameter in particular, coefficient of correlation (R) values of 0.883 [95] and 0.890 [94] were observed. By using FT-near infrared reflectance spectra, coefficient of determination (R^2) values up to 0.819 for the thick albumen height [104] and 0.722 for the air cell height [104] were obtained.

Narushin et al. [108] reported the comparison between infrared spectroscopy (IRS) and egg size measurements for the non-destructive evaluation of eggshell strength. Infrared and egg size parameters were correlated with eggshell quality parameters as obtained using invasive techniques. A correlation coefficient of 0.72 demonstrated the ability of IRS to predict shell weight with reasonable accuracy. Another study was done by Aboonajmi et al. [109] to evaluate poultry egg freshness using VIS/NIR spectroscopy. In this study, a new method for egg freshness prediction using transmission visible near-infrared spectroscopy was investigated. The non-destructive VIS/NIR spectral measurements from 300 to 1100 nm (832 wavelength) were used as well as Haugh units and air cell height for each egg, and a database was created for both environments. Finally, a maximum likelihood latent root regression algorithm was developed to predict Haugh unit and air cell height by the observed spectrum. The results showed that this method was better in comparison with partial least square regression (R^2 up to 0.79 and 0.72 for air cell height and Haugh unit).

Aboonajmi et al. [110] carried out a quality assessment of poultry eggs using VIS/NIR spectroscopy and radial basis function networks. The developed models yielded a good prediction accuracy of Haugh unit for intact egg (R^2 values 0.745 and 0.76) as well as air cell height (R^2 values 0.835 and 0.844) for room and refrigerator conditions, respectively [110]. The results of the experiment showed that the developed model can be used to predict egg freshness indices satisfactorily.

The potential of the ultraviolet and visible (UV/VIS 200–800 nm) transmittance method to inspect the internal quality (freshness) of intact chicken egg was investigated by Liu et al. [111]. It was concluded that the non-destructive inspection of egg freshness by transmittance properties is feasible in the range of 400–600 nm, while it is impossible to inspect egg freshness in the range of 200–400 nm due to the low transmittance.

Giunchi et al. [112] evaluated the freshness assessment of shell eggs using FT-NIR spectroscopy non-destructively. Diffuse reflectance spectra were acquired in the spectral range 833–2500 nm on the samples of eggs collected from three different farms and characterized by different methods. Models were performed, and the prediction showed R^2 values up to 0.722, 0.789, and 0.676 for air cell height, thick albumen heights, and Haugh unit, respectively (test set validations). The specimens were correctly classified (100%) according to the days of storage. Hierarchical cluster analysis showed a low level of heterogeneity for the three previous methods within the days of storage.

Another study was carried out by Abdel-Nour et al. [113] to predict the egg freshness and albumen quality by means of visible/near-infrared spectroscopy as a rapid and non-destructive technique for egg quality assessment (Figure 12.4). The

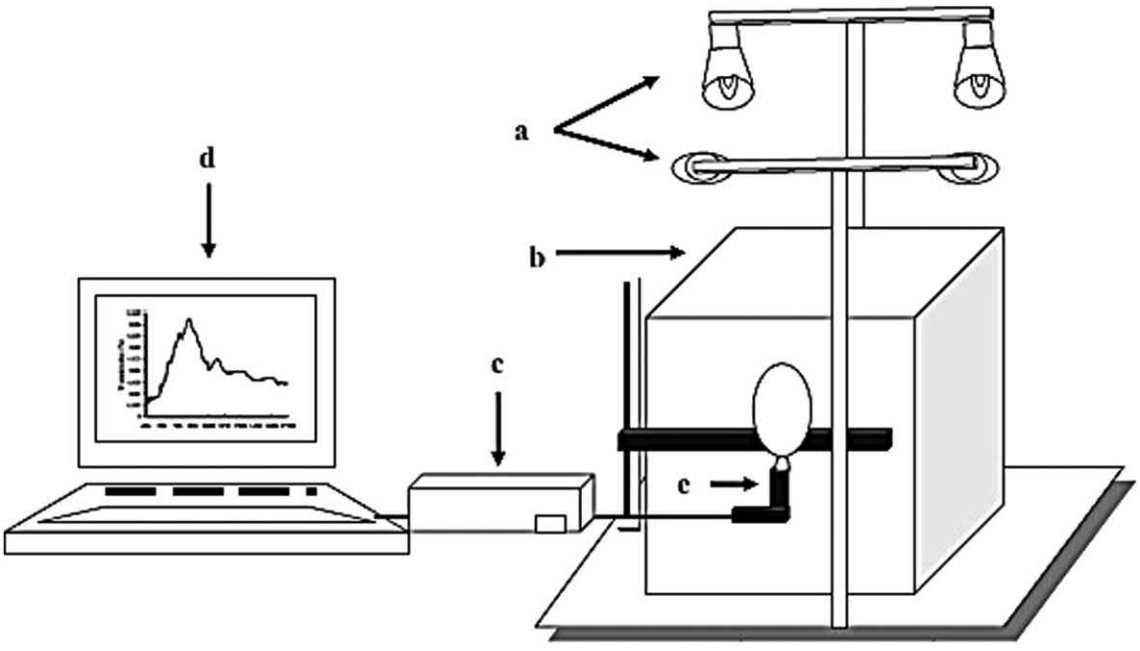

FIGURE 12.4 System setup: (a) halogen lighting source, (b) white frame, (c) spectroradiometer, (d) personal computer, and (e) sensor. (From Abdel-Nour et al. [113].)

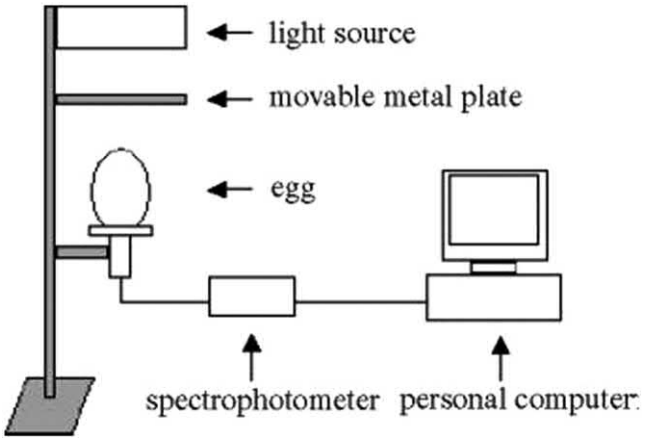

FIGURE 12.5 Experimental set-up for the measurements of transmission spectra. (From Kemps et al. [114].)

results showed that VIS/NIR transmission spectroscopy acts as a useful tool in assessing egg freshness and albumen pH and can be suitable for use as a non-destructive method to predict HU, albumen pH, and length of storage.

Kemps et al. [114] investigated the feasibility of visible transmission spectroscopy for freshness assessment of an individual egg (Figure 12.5). The non-destructive spectral measurements were compared with the two most widely used destructive freshness parameters, namely Haugh units and albumen pH. A model of partial least squares (PLS1) was performed to predict Haugh units and pH of the albumen. These results showed that the light transmission spectrum of an egg provides quantitative information about egg freshness [114].

Another property used to predict physical features is dielectric properties. Materials' dielectric properties are influenced by the electric conduction, dipoles, electronic, and ionic properties. Maxwell–Wagner mechanisms are closely dependent on electromagnetic wave frequency [115]. This is useful in studying and developing heating processes or grading techniques based on electromagnetic energy.

Several studies have been performed to characterize the dielectric properties of foods over the past 50 years [116–119]. Dielectric properties are related to temperature and frequencies, and these properties have been reported for different agricultural commodities, including grains and seeds [120], fruits and vegetables [121–129]. Studies also reported the possibility of non-destructively predicting selected physical characteristics of foodstuffs, like fruits and eggs [2, 130, 131]. No similar studies have been reported that relate dielectric properties of egg ingredients to storage quality. Changes in the dielectric parameters of fresh eggs during storage were investigated for the development of a method to assess the main quality indices by Ragni et al. [132]. The dielectric constant and loss factor of yolk generally increased with the storage time.

A simple resonant radio frequency circuit equipped with a parallel plate capacitor probe was used to predict the qualitative indices of shell eggs in other studies [133]. For hatching eggs, shells have to be thick and strong for the preservation of the embryo as well as thin for gas exchange and weak enough to allow the chick to crack the shell when hatching [134]. For maximum efficiency, it is important to detect eggs with unfit shells and remove them from further incubation, processing, and/or transportation. Shell-breaking force and/or shell stiffness, calculated as a ratio of force and shell deformation at failure, can adequately determine shell strength characteristics directly [58, 59, 135–137]. It was shown that such shell thickness, the ratio of shell weight to surface area, and the percentage shell are in close relationship to the shell-breaking force and shell stiffness [58, 59, 138–143]. Therefore, it is possible to predict the strength characteristics of eggshells indirectly. However, as all these parameters are destructive, there are no values for practical application. The following non-destructive parameters are used to indirectly predict shell characteristics: egg specific gravity [54, 58, 70, 139, 143, 144], egg mass [145–147], and shell non-destructive elastic deformation [17, 59, 97, 144, 148]. However, none of the above variables can accurately predict the strength characteristics of the shells [149, 150]. The correlation of shell-breaking force with egg weight was found to give a value for the correlation coefficient (R) of 0.27; 0.04 value for specific gravity of egg [150]; and with non-destructive elastic deformation, the value for R was 0.47 [150].

The relationship between shell compressive stress and the non-destructive elastic deformation was improved by considering additionally the values of egg length and maximum breadth [151]. Several studies reported the possibility of using infrared spectroscopy (IRS) to define the quality of foodstuffs like meat [152], food [153], and animal feeds [154, 155]. The infrared spectra absorption is proposed for the relation of the shell and its density, inner structure, and/or chemical composition, and so correlated with the shell strength. Guo et al. [156] surveyed the dielectric properties of the albumen and yolk of eggs at 24°C over the frequency range from 10 to 1800 MHz to monitor quality changes during a 5-week storage period at 15°C (Figure 12.6). Quality factors including Haugh unit, yolk index, moisture content, and egg weight were also measured during the same period [157]. On average, the

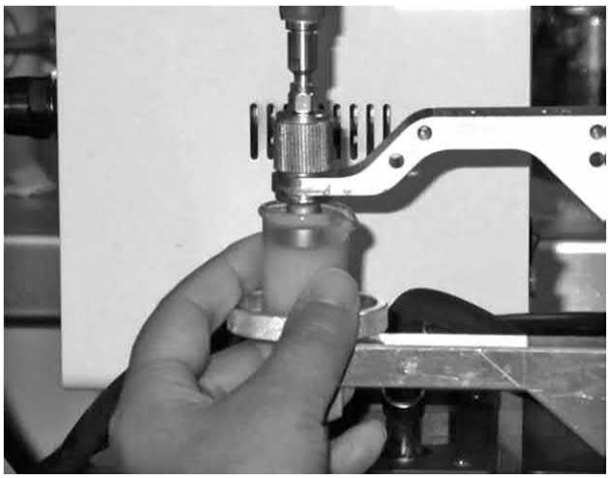

FIGURE 12.6 Dielectric properties measurement arrangement. (From Guo et al. [156].)

Haugh unit, yolk index, and egg weight decreased with time, indicating its quality deterioration. Albumen moisture content increased at first and then decreased after the third week of storage. The opposite trend was observed for the yolk moisture content. Correlations between these quality changes and corresponding measured dielectric properties over the storage period show potential for the use of dielectric measurement techniques for the rapid assessment of egg quality.

As mentioned above, by correlating measured dielectric properties to physical properties of interest, a line of metrology was developed with a wide spectrum of applications including pharmaceuticals, mining, construction materials, food, and agriculture [158–160]. The most popular and well-known applications are moisture sensing [161], microwave heating [162], and remote sensing [163, 164].

Recent developments in electronic nose (EN) technology and modern statistical methods including chemometrics and artificial intelligence provide opportunities to extend the scope of odor measurement. This new technology, the electronic nose, is an instrument including an air sampling apparatus and an array of gas sensors interfaced to a personal computer (PC) or an embedded system. The most important feature that distinguishes an electronic nose from other instruments is the ability of its sensor array to respond differently to various odors. Each odor may contain hundreds, sometimes thousands, of different volatile organic compounds (VOCs). Classical spectrometry analytical methods such as gas chromatography–mass spectrometry (GC–MS) are able to identify and measure individual odorous chemical compounds in an odor sample. On the other hand, the electronic nose can react to the "total odor sample" as does a human nose. In the human olfactory sensing system, it is not necessary to separate individual chemicals in the sample as part of an assessment process; the odor is assessed as a whole, and the odor is identified using our brain (i.e. the odor pattern is recognized from the memory bank).

Researchers have identified that the electronic nose is capable of quantifying odors and discriminating between odors from different sources [165]. Developments in the statistical techniques required to analyze outputs from electronic nose devices have broadened the scope of electronic nose applications. Bicego et al. [166] identified how an apparent lack of reproducibility of the sensors could be compensated for by using a flexible calibration and recognition tool based on neural networks. Boccorh et al. [167] postulated that the predictions made with artificial neural networks (ANN) arose from the ability of neural networks to simulate the non-linear relationships observed in human perceptions such as taste and odor. Improved statistical methods were used by Guadarrama et al. [168] to discriminate between various VOCs derived from car components. More recently, Sohn et al. [169–171] and Qu et al. [172] were able to determine significant relationships between electronic nose output and odor concentration determined by dynamic olfactometry. The former researchers were then able to accurately determine the odor concentration of samples to train the electronic nose using an ANN approach. Although errors associated with olfactometry were identified as a constraint to improve the accuracy of electronic nose odor prediction model, it was shown that ANN algorithms significantly improved the model's ability to predict new samples when compared to alternative linear and non-linear multivariate modeling techniques. Sohn et al. [170] also showed that an electronic nose with a reduced number of sensors could quantify odor concentrations from a specific source. In these approaches, reliable olfactometry data are important because ANNs are trained using not only the sensor outputs of an electronic nose but also the odor concentration results from olfactometry. The air quality research group in the Department of Primary Industries and Fisheries, Queensland (QDPI&F) has developed and evaluated an electronic nose system. This system includes an array of metal oxide semiconductor (MOS) sensors, which are appropriate for the assessment of odor emissions from intensive livestock industries. This was due to their sensitivity to volatile chemicals found in such odors. The electronic nose is able to provide qualitative information (i.e. discriminate between samples from different sources) and predict odor concentrations using a model based on results from olfactometry. This device and associated models were applied to odors derived from a broiler shed.

A sample study performed by Dutta et al. [173] on egg freshness considered an electronic nose-based approach (Figure 12.7). Principal component analysis, fuzzy C means, self-organizing maps, and 3D scatter plots were used to define regions of clustering in multi-sensor space according to the state of freshness of the eggs. These were correlated with the "use-by date" of the eggs. Then four supervised classifiers, namely multilayer perceptron, learning vector quantization, probabilistic neural network, and radial basis function network, were used to classify the samples into the three observed states of freshness. A comparative evaluation of the classifiers was conducted for this application. The results showed that this method was able to predict egg freshness into one of three states up to 95% accuracy with good potential for commercial exploitation. Sohna et al. [174] implemented an electronic nose for continuous odor monitoring in a poultry shed. The results demonstrated that it was possible to develop a model to allow an electronic nose to provide a semi-continuous measurement of odor concentrations. The electronic nose

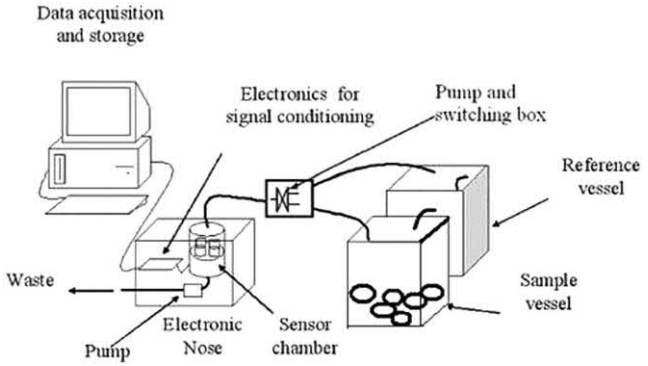

FIGURE 12.7 Experimental set-up for egg quality monitoring experiments. (From Dutta et al. [173].)

was also able to demonstrate the influence of shed conditions on odor emissions.

12.2.4 Imaging Techniques

Computer vision techniques are carried out in three main applications for egg grading purposes. First, a computer vision system needs to be developed to detect open cracks and hair cracks in eggs. The first application was reported by Elster and Goodrum [175]. Based on their work, Patel et al. [176] increased the detection rate to nearly 90% using a decision algorithm based on neural network development. Instead of neural network analysis, Han and Feng [177] used a two-dimensional fast Fourier transformation to extract relevant information from the images, but their detection rate was not more than 88%. Bloodspot detection was also performed using computer vision. Using a color image analysis combined with a neural network detection technique, 92.8% of the bloodspots in eggs could be detected.

Dirt stains can be detected in different ways, as reported by Patel et al. [178], Garcia-Alegre et al. [179–182], and Ribeiro et al. [183]. Recently, new research has shown the possibility of differentiating between the different types of dirt that can be found on the egg (feces, uric acid, yolk, and blood). This was done with high accuracy and a rather low computing time, making on-line implementation possible [184]. A blood spot detection neural network was trained, tested, and evaluated entirely on eggs with blood spots and fresh (grade A) eggs [185]. The neural network could accurately distinguish between fresh and blood spot eggs. However, when eggs with other defects were included in the sample, the accuracy of the neural network was reduced. The accuracy was also reduced when evaluating eggs from other poultry houses. To minimize these sensitivities, eggs with cracks and dirt stains were included in the training data, for example, eggs without blood spots. The training data were also combined with eggs from different sources. Similar inaccuracies were observed in neural networks for crack detection and dirt stain detection. New neural networks were developed for these defects using the method applied for the blood spot neural network development. The neural network model for blood spot- and dirt-stained egg detection had an average accuracy of 92.8 and 85.0% respectively. The average accuracy of the crack detection neural network was 87.8%. These accuracy levels were sufficient to produce graded samples that would exceed the USDA requirements (Figure 12.8).

Gittins [186] studied alternative methods to grade eggs and developed an electronic system for measuring various characteristics of an egg, such as weight, color, albumen quality, yolk color, and shell density. Elster and Goodrum [187] developed a program to analyze grayscale images of stationery eggs for cracks. The egg was isolated from background noise and enhanced using image processing algorithms. A 96% success rate was achieved. However, the average time required to process one egg was 25.3 seconds. Goodrum and Elster [188] extended their work to detect cracks at any point on the surface of rotating eggs. The identification of cracks

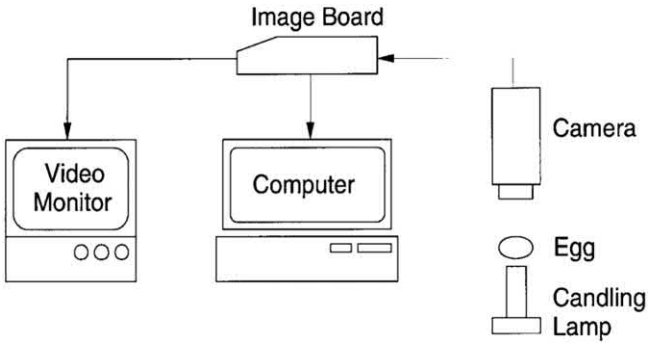

FIGURE 12.8 A schematic diagram of the imaging system. (From Patel et al. [185].)

was dependent on the egg size and required software calibration constants. Bullock et al. [189] provided a brief tutorial on artificial neural networks and discussed two applications—the inspection of cookies for damage, and inspection of apples for bruises. The use of artificial neural networks and color computer vision systems in agriculture were discussed by Davidson and Lee [190]. However, various application areas and potential uses, such as planning, harvesting, sorting and inspection, image analysis, and the control of processing plants, were outlined [190–192].

Patel et al. [185] used image acquisition routines to capture grayscale images of cracked and fresh (grade A) eggs. Histograms of the images were generated and used to train a neural network for the detection of cracked eggs. The model was 90% accurate and provided significant improvement in speed over the method of Elster and Goodrum [186]. The work was extended to the detection of blood spots and dirt stains. The neural network model for blood spot detection was 85.6% accurate. An accuracy of 80% was achieved on dirt stain detection.

Grading and quality inspection of defective eggs using machine vision were performed by Dehrouyeh et al. [193]. They showed that the algorithms were based on image processing to detect internal blood spots and eggshell dirt by processing acquired images from eggs under different illuminations. The algorithm can also detect the severity of dirt on an eggshell. In order to carry out image processing and extract useful features of captured images of eggs by machine vision, an algorithm in HSI color space was developed. The results of the experiments indicated that the accuracy of differentiation of the blood spots algorithm was 90.66% of defective eggs and 91.33% of intact eggs, while the total average of this algorithm was 91%. Accuracy of differentiation of the dirt-detect algorithm was 86% of clean eggs, 83% of low-dirt eggs, and 88% of high-dirt eggs. The total average of this algorithm was 85.66%.

12.3 POSTHARVEST HANDLING OF FRESH POULTRY EGGS

12.3.1 Transport and Shipment

Several stress conditions can produce deterioration in the internal characteristics of eggs, such as vibrations and impacts due to shipment. From the research carried out by

Adam and Skinner [194], the Haugh unit measurements were significantly influenced by the container position, as they were probably subjected to different temperatures, air movement, and vibrations on the truck. A significant decay in the albumen and yolk quality of eggs following journey hazard tests, such as vibration, impact, and drop, was reported [195, 196]. The road roughness, distance, and traveling speed, load, some characteristics of the truck such as the suspension and the number of axles influence the vibrations due to transportation [197, 198]. The effects of the transportation on foodstuffs depend on the type of packaging [199]. Some types of packaging, such as bulk bins, can remarkably amplify vibrations during transportation from the bottom to the top of the shipment column [200–202]. In order to assess the effects of transport strains on foodstuffs, several studies were performed by reproducing conditions in the laboratory, and power spectral density (PSD) curves were calculated from the vibrations measured during transportation. For this purpose, a shipping column containing the produce was attached to the vibrating table of an electro-hydraulic or electro-dynamic shaker, which could simulate the transport conditions [203, 204]. The effects of transport on foodstuffs were also assessed by vibration tests according to the ASTM standards [ASTM, 1979, ASTM, 1987, 205, 206]. Berardinelli et al. [207] reviewed the effects of transport vibrations on the parameters describing the egg quality: Haugh unit, vitelline membrane strength, and air cell height (Figure 12.9). Measurements of mechanical vibrations were carried out on the floors of three vehicles during the transportation of eggs from the packing house to the market. These transport vibrations were used to stress a typical packaging column for egg distribution by means of an electro-dynamic shaker. Haugh unit (28%) in the sample with vibration showed the highest level as compared to a non-vibrated sample when analyzed by PSD. Variations in the resistance of the vitelline membrane (18%) were also observed among the samples which were vibrated with different PSD profiles.

12.3.2 Coating

Over recent decades, a number of studies have been performed on the use of edible films and coatings. They have taken the form of moisture, gas, or solute barriers in food packaging to extend the shelf life and to improve overall quality. Edible films and coatings from biopolymers have received attention. Potential sources and applications of edible films and coatings have been reviewed by Guilbert et al. [208], Debeaufort et al. [209], and Morillon et al. [210]. The most plentiful organic renewable resource is cellulose, a polysaccharide composed of linear chains of (1–4)-b-D-glucopyranosyl units; its derivatives have unique physical, chemical, and colloidal properties, and an ability to form films. They have been used as edible films and coatings since the 1980s [211].

Cellulose-based materials are very efficient oxygen and hydrocarbon barriers, and their water vapor barrier properties can be enhanced by the addition of a lipid [212–214]. Fresh eggs are an excellent protein source. Nevertheless, several problems, such as weight loss and interior quality deterioration

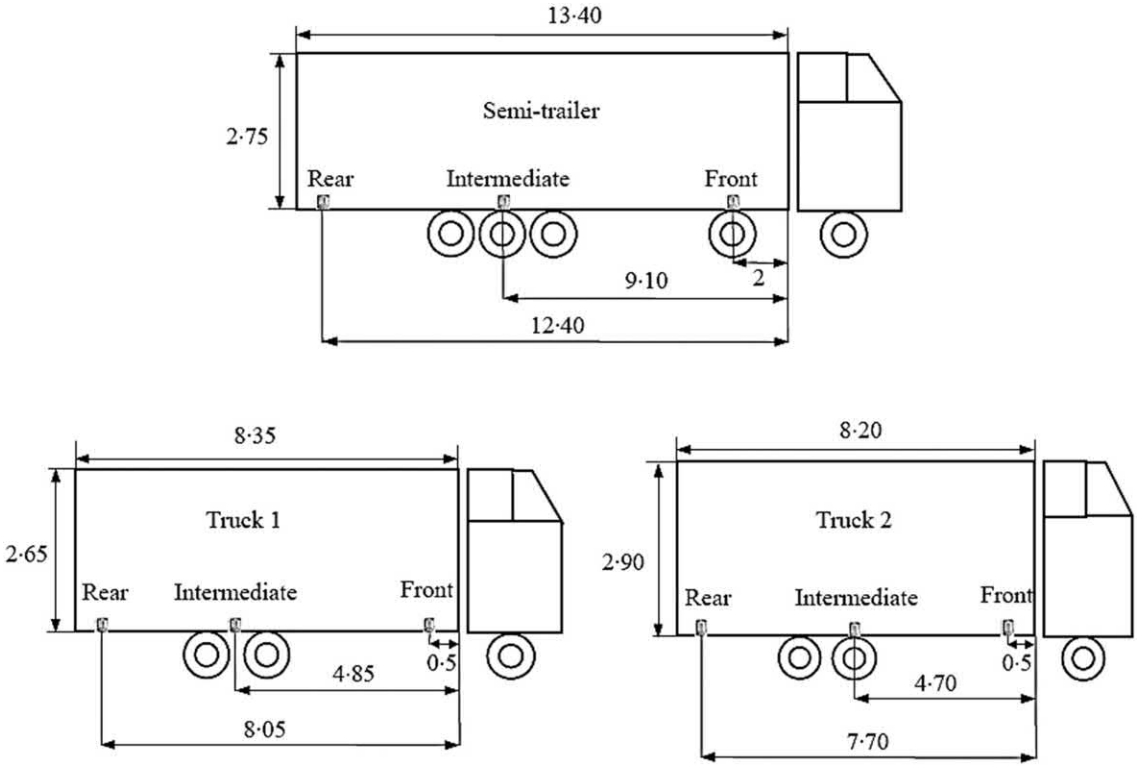

FIGURE 12.9 Geometrical characteristics of the vehicles and positions of the transducers on the floor (all measurements are in m). (From Berardinellu et al. [207].)

encountered during egg storage, have caused major economic losses to the poultry industry [215]. During the storage of eggs, quality deterioration is directly associated with changes in egg white (albumen), yolk, weight, and pH. Meyer and Spencer [216] reported the effects of various coatings, including polyvinyl alcohol, acrylic resin, and zein, on shell strength and egg quality. Herald et al. [217] studied the quality of eggs coated with wheat gluten solution. In a study by Bhale et al. [218], the efficacy of chitosan coating in improving the shelf life of eggs was reported. In addition, Xie et al. [219] stated that eggshells coated with soy protein isolate (SPI), whey protein isolate (WPI), carboxy methyl cellulose (CMC), or wheat gluten (WG) showed greater puncture strength than those of non-coated eggshells. The results showed that edible coatings enhance eggshell breakage minimization. In terms of the bacterial barrier property, it was found that all treatments with these four different coatings significantly reduced post-wash dye penetration into eggs (water, NaOCl, or Na_2CO_3 washed only).

Suppakul et al. [220] reported a methylcellulose (2.00% w/v) and hydroxypropyl methylcellulose (1.00% w/v) based coating was formulated to study the effects of polyethylene glycol-400 (PEG-400). They used a stearic and palmitic acid blend (SPB) on water vapor permeability (WVP) and tensile properties. The efficacy of cellulose-based coating on fresh egg quality during 28-day storage at ambient temperature was investigated in terms of weight loss, pH, and albumen quality. An edible cellulose-based solution was prepared for eggshell coating. A batch of fresh, grade AA 1-day eggs was coated with the cellulose-based coating solution. The other batch consisted of uncoated eggs, which served as the control. This study highlighted the promising use of a cellulose-based coating to enhance the shelf life of fresh eggs.

Another attempt at coating was assessed by Kim et al. [221]. They evaluated the chitosan–lysozyme composite coating on hard-boiled eggs, which exhibited antimicrobial control against *S*. Enteritidis on shell-on eggs. They proposed that chitosan–lysozyme-based coatings may be used to increase microbial safety and extend the shelf life of hard-boiled eggs by reducing post-processing contamination.

12.3.3 Storage Temperature

Chung and Lee [222] sampled eggs from two flocks of different ages (40- and 60-week-old laying hens) in a commercial layer operation immediately after laying and used them for egg quality testing after periods of storage of 7, 14, 21, and 28 days *at* room and refrigerator conditions [222]. Freshly laid eggs from the two age groups did not differ in egg weight, Haugh unit, albumen pH, albumen and yolk weights, or eggshell thickness. Upon incubation at different temperatures and durations of storage, the age of hen did not affect egg weight loss or Haugh unit, but affected yolk and albumen weights. The increase in storage temperature accelerated egg weight loss and reduction in albumen weight but decreased Haugh unit and the pH of albumen. As the storage duration increased, egg weight loss, albumen pH, and yolk weight were increased, but the Haugh unit was lowered. Interaction between temperature and storage duration was noticed in egg weight loss, Haugh unit, and albumen pH. It is concluded that egg quality is more affected by temperature or storage duration, but not by hen age. As the age of hens is an important contributing factor to egg quality, further study is needed to clarify the lack of clear effect of hen age on egg quality which emerged from this study.

12.3.4 Washing Process

Egg washing is currently not permitted within the European Union, with few exceptions. This is mainly because there are concerns that cuticle damage could occur during or after the washing process, as a result of a suboptimal operation. Leleu et al. [223] compared the cuticle coverage levels of 400 washed and unwashed eggs, derived from either a brown or a white egg-laying flock at the end of lay. The eggs from older hens inherently have poorer cuticle coverage and as a result constitute a greater risk to safety when eggs are washed. Thus, the effects of the washing procedure used in this study on cuticle quality were tested under the worst-case scenario. A standard Swedish egg-washing process was used. The cuticle coverage of the eggs was assessed by a colorimeter by quantifying the color difference before and after staining with Tartrazine and Green S. The cuticles of an additional 30 eggs from each of the four groups were then visually assessed by scanning electron microscopy. The staining characteristics of the cuticle varied greatly within each group of eggs and showed that the washing process did not lead to cuticle damage. Scanning electron microscopy confirmed that there was no irreversible damage to the cuticle of the washed eggs and that it was not possible to correctly assign the treatment (washed or not) based on a visual assessment. In conclusion, no evidence could be found to suggest that the washing procedure used in this investigation irreversibly changed the quality of the cuticle.

Chemical washing also affects the microstructural changes of the eggshell [224]. Cetylpyridinium chloride (CPC) solution at 10, 50, or 100 ppm, and trisodium phosphate (TSP) at 0.5, 1.0, or 5.0% were evaluated and eggshell surfaces were examined using scanning electron microscopy (SEM). As the concentration of CPC increased, the eggshell surface became pitted and the cuticle layer became thinner. TSP caused the cuticle layer to fissure and flake, and even cuticle-free areas were observed at 5.0%. When the porosity of eggs was measured using the blue lake staining method, there were significant differences between control and TSP- or CPC-washed eggs as observed by SEM. These results suggest that depending on the types of chemicals used in the wash water, different microstructural changes occur in eggshell surfaces, and the more damaged eggshell surfaces allow more bacterial penetration.

12.3.5 Packaging

Zabaniotou and Kassidi [225] presented the application of life cycle assessment (LCA) for the comparison of two egg

packages, from polystyrene and recycled paper. The input and output streams of mass and energy were examined, and the environmental impacts associated with the two systems were analyzed. The application of LCA using Eco-Indicator 95 has made possible the comparison of the environmental impacts of two egg packages. The results of this LCA study are discussed and it is revealed that the PS packages contribute more to acidification potential, and winter and summer smog, while recycled paper egg packages contribute more to heavy metal and carcinogenic substances impact. Nevertheless, it seems that paper egg packages have less environmental impact than polystyrene ones with the assumption that the accuracy of the results is limited by the credibility of the European databases used for primary data [225].

12.4 CONCLUSION

The demand for fast, non-destructive, and reliable egg quality assessment techniques motivated the development of the acoustic or optical method. However, promising results are available from the lab-scale experiments, but commercial applications did not emerge significantly [18, 19]. The classic approaches like measuring Haugh units or shell breaking force are destructive tests and not suitable for large-scale application. The dynamic stiffness of an egg or optical absorption at a given wavelength is still not in use. However, it might not be good practice to always mimic the older destructive methods such as breaking strength with a new technique and report results in terms of the older standard units. This is certainly the case if the old technique measures something that is only moderately correlated with the desired characteristic (e.g. non-destructive deformation as related to the number of broken eggs in practice). If, however, the old reference is in principle a good measure of the desired characteristic but is time-consuming and/or destructive, then mimicking those older reference methods with new, fast, and non-destructive methodology can still be of interest.

A fast and non-destructive quality assessment tool together with modern information technology has multiple advantages that reach far beyond the classical grading step in the total egg production chain. In the packing plant, such fast technologies allow for measuring the quality of an individual egg instead of estimating the quality by sampling some eggs out of a large batch. This allows for a very concise and rapid tracking of batch quality. Moreover, these techniques allow for measuring more quality attributes and thus can guarantee a better overall quality of each individual egg. The data that are gathered from such packing plants about several quality attributes can be fed back to egg producers by means of modern information technologies. This allows the producer to track the quality of the delivered eggs, and eventually to trace anomalies within hours. As an example, it is proven that the dynamic stiffness of an egg is impaired by heat stress.

It has been shown that a long list exists of techniques that can be used to grade eggs. Some of them are used already on commercial grading machines. The possibilities of other techniques have to be explored more in detail before they become of real commercial value. The price to implement other techniques is too high or the computer time needed for the measurements is too long, and hence more development is needed to solve these problems. An interesting idea for the scientists involved in the development of these techniques is to use combinations of different non-invasive techniques to define the risk that an egg can be dangerous for the consumer's health. Before this will be possible, we have to be able to make more links between the inner content of the egg and the bacterial defense mechanisms present in the egg. Another challenging concern which leads to economic losses of poultry eggs is damage caused by vibrations and impacts during shipment. The road roughness, distance, and traveling speed, load, and some characteristics of the truck influence the vibrations due to transportation.

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Part II

Preservation of Minimally Processed Foods

13 Minimal Processing of Fruit and Vegetables

Conrad O. Perera

CONTENTS

13.1 INTRODUCTION

In recent years, the demand for high-quality fruit and vegetables has increased considerably around the world due to the health consciousness of consumers. Minimally processed fruit and vegetables (MPFV) possess fresh-like quality characteristics of flavor, texture, color, and aroma, and these are convenient to use [1]. Other terms used for the minimally processed produce are fresh-cut, lightly processed, partially processed, fresh processed, and pre-prepared. MPFVs are also important to the foodservice industry, such as restaurants and catering companies, as they offer many advantages (i.e. convenience, low cost, savings in labor, and high hygiene levels) over traditional products. Despite its popularity, the production of minimally processed products is limited due to their rapid deterioration and senescence (natural aging leading to death of the tissues). Hence minimally processed produce is more perishable than its unprocessed raw materials [2], since nutrients are easily available to the microorganisms, and reactants are more prone to interact with each other.

The minimal processing of fruit and vegetables generally involves washing, peeling, slicing, or shredding before packaging and storage at low temperatures. All these steps have an effect on the nutrients, shelf life, and quality of the prepared products [2, 3]. Examples of these products already on the market include packaged shredded lettuce/cabbage/carrots, cut fruit and vegetable salads, and peeled/sliced potatoes/carrots, and broccoli and cauliflower florets. More recently, tropical fruits such as peeled, cut, and sliced mango, pineapple, papaya, watermelon, jackfruit, and durian segments have appeared on the market as minimally processed products [4, 5].

Minimal processing of raw fruit and vegetables should serve two purposes. The first purpose is the importance of freshness of the products with nutrition quality and convenience of use. Second, the product should have a shelf life sufficient to make distribution feasible within the region of consumption. The shelf life of minimally processed fruit or vegetables, in terms of microbiological, sensory, and nutritional quality, should be at least 4–7 days, but preferably even longer. The producers and distributors demand increased

product safety. MPFV products are highly perishable, thus preservation actions are important in making the products safe. Technologies that allow for a two- or three-fold extension of the shelf life are important to reduce the food losses. In order to produce minimally processed fruit and vegetables with a longer shelf life, good safety considerations, cleaning, peeling, cutting, and packing are usually carried out in centralized locations.

Minimally processed produce is often less stable due to the enzymatic activity of the cut cell walls and also to bacteriological contamination from handling during treatment. Various postharvest treatment methods are employed to increase the biological stability and to extend the shelf life of products. In all these treatments, low temperatures and good hygiene practices are essential to maintain food safety and to achieve the desired shelf life.

Contamination of fruit and vegetables in the field and unhygienic handling practices are sources of human infection and illness. Irrigation water must not be contaminated, raw manure should not be used as fertilizer, and sanitation facilities should be provided for field workers. The trucks used for transportation should be cleaned especially if animals are also shipped in them. The use of clean ice and refrigerated trucks is recommended. Once produce is harvested, the dirt should be cleaned off with water maintained at the proper temperature to prevent contamination [6]. The use of chlorinated water (200 ppm chlorine) in the packing line is recommended. For MPFVs that are usually eaten raw, there is no treatment that can be relied on for the total elimination of the contaminating microorganism. However, the risks of contamination could be minimized by adhering to Good Manufacturing Practices (GMP) and food safety plans based on hazard analysis critical control points (HACCP) [6].

13.2 PHYSIOLOGICAL RESPONSES AND BIOCHEMICAL CHANGES

Fruit and vegetables are living organs of plants that continue to respire after harvest. Plant tissues incur damage during minimal processing but remain raw and still living after processing. The physiology of MPFVs is essentially the physiology of wounded tissue. This type of processing, involving abrasion, peeling, slicing, chopping, or shredding, differs from traditional thermal processing. In the case of MPFVs, the tissue remains viable during subsequent handling. Thus, the behavior of the tissue is generally similar to that of plant tissues that have been wounded or exposed to stress conditions [7].

The rate of respiration and ethylene production markedly increases within minutes of undergoing minimal processing of fresh produce, and essentially a "wound response" is initiated [7]. Both respiration and ethylene production can result in a shorter shelf life for the product. The ethylene can accelerate ripening, softening, and senescence [8], which leads to membrane damage, while the respiration uses energy reserves. Other consequences of wounding are chemical and physical in nature, such as oxidative browning reactions and lipid oxidation or enhanced water loss [7].

Injury-stresses caused by minimal processing result in mechanical rupture of tissues, and cellular decompartmentalization leading to delocalization and intermixing of enzymes and substrates. One such enzyme system is the ascorbic acid oxidase, which oxidizes ascorbic acid to dehydroascorbic acid, which can then further degrade to other compounds leading to browning. Thus nutritional quality such as vitamin C is lost [9]. Therefore, wound-induced physiological and biochemical changes take place more rapidly than in intact raw commodities, and microbial proliferation may be accelerated.

There is little information about the physiology and chemistry affecting minimal processing of tropical fruit and vegetable products. Such information is vital for the extension of the marketing of both fresh and minimally processed products. Pineapple, which because of its morphology is difficult to consume directly, stands out among the tropical fruits that have the potential to be commercialized as ready-to-eat products [10–12]. Recently, ethylene receptor inhibitors such as 1-methylcyclopropene (1-MCP) that retard C_2H_4 biosynthesis have been tested on guavas [13], mangoes [14], and other tropical fruits to extend the shelf life with success [15].

Tropical fruits such as jackfruit, durian, and pineapple are ideal for minimal processing because of their large size and difficulty in peeling. Minimal processing can also ease export by reducing air-freight cost and quarantine barriers, which are related to the compliance with regulations related to fruit pests, particularly fruit flies. Recently it was reported in the International Tropical Fruit Network, about Malaysian exports of minimally processed tropical fruits to various European countries [ ].

13.2.1 Ethylene

Ethylene is a naturally occurring plant hormone that has numerous effects at very low concentrations ($\mu l L^{-1}$) on the growth, development, and storage life of many fruit and vegetables, and ornamental crops [16]. It is produced virtually by all parts of the higher plants, including leaves, roots, flowers, fruits, tubers, and seedlings. The pathway of ethylene biosynthesis elucidated by Adam and Young [17] is now well-documented, and in both wounding and ripening, the pathway is the same. Methionine is first converted to S-adenosyl methionine (SAM) that then gives rise to 1-aminocyclopropene 1 carboxylate (ACC), catalyzed by ACC synthase. The final step is catalyzation by ACC oxidase (also known as the ethylene-forming enzyme), in which ACC is converted to ethylene. In most plant tissues, the level of active ACC synthase determines the rate of ethylene production; however, the mechanism(s) underlying the regulation of ACC synthase gene(s) during plant development is unknown. Both ACC synthase and ACC oxidase transcript levels greatly increase due to ripening and wounding [16]. The biosynthesis pathway is illustrated in Figure 13.1.

Harvested fruits and vegetables may be intentionally or unintentionally exposed to biologically active levels of ethylene, and both endogenous and exogenous sources of ethylene contribute to its biological activity [16]. Figure 13.2 is a

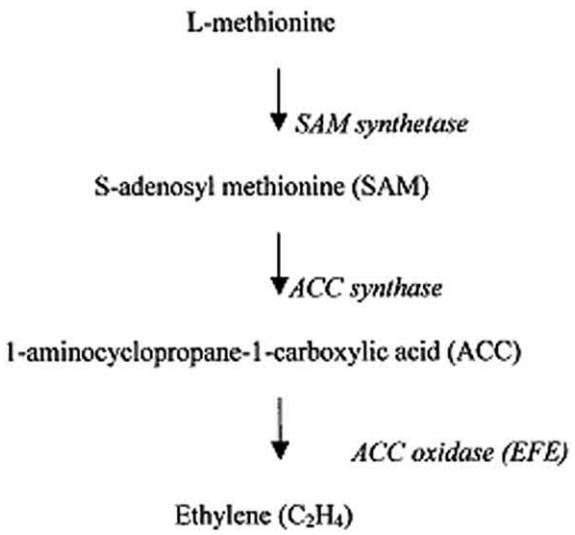

FIGURE 13.1 Simplified diagram of ethylene production in higher plants. (Adapted from Adams and Young [17].)

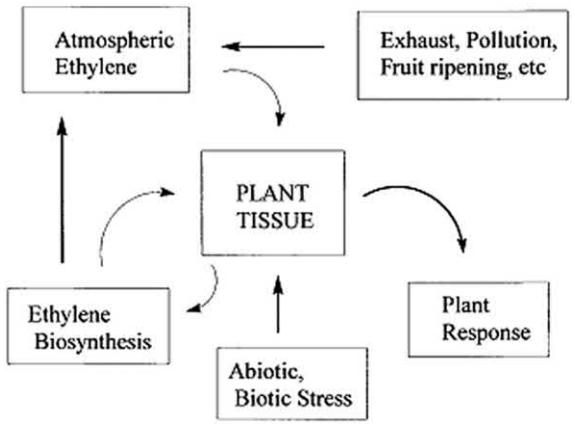

FIGURE 13.2 Ethylene interactions with the plant and its environment. (Adapted from Saltveit [16].)

schematic diagram showing the ethylene interactions between plants and their environment. Endogenous sources of ethylene originate from internal synthesis within plants and fruits, and exogenous sources are from external sources such as engine exhaust, heaters, or ripening fruits. Ethylene production is promoted by stresses such as chilling injury [18] and wounding [19], and this stress-induced C_2H_4 can enhance fruit ripening. Cell wall enzymes, such as exo- and endo-polygalacturonase, β-galactosidase, and pectinmethylesterase, induced by ethylene can digest cell walls, resulting in texture changes [20], and lipoxygenase can degrade membrane lipids [21].

Wounding plant tissues induces elevated ethylene production rates, sometimes within a few minutes, but usually within 1 hour, with peaks usually within 6 to 12 hours [19]. Ethylene produced by the physical action of minimal processing was found sufficient to accelerate softening of banana and kiwifruit and chlorophyll loss in spinach [22]. The ethylene level increases in proportion to the amount of wounding in several fruits and vegetables [7]. Levels of ACC and ACC synthase activity increase the ethylene in tomato, winter squash, and cantaloupe muskmelon [19].

There are various detrimental effects of ethylene on fruits and vegetables. C_2H_4 can cause yellowing of green stem and leafy vegetables [16]. Ethylene from either endogenous production or exogenous applications stimulates chlorophyll loss and the yellowing of harvested broccoli florets [23]. Russet spotting is a postharvest disorder of lettuce in which small brown sunken lesions appear on the leaf. It is caused by the exposure to hormonal levels of C_2H_4 at storage temperatures of around 5°C [24]. Many biotic and abiotic stresses stimulate phenylpropanoid metabolism and the accumulation of phenolic compounds in lettuce [25]. However, even though the level of phenolic compounds is elevated in stressed lettuce, ethylene is still essential for the browning reaction, which is characteristic of the occurrence of russet spotting [26]. The firmness of many ripening fruits and vegetables decreases with C_2H_4 treatment. This is usually beneficial when associated with ripening (e.g. bananas, tomatoes), but if applied for too long, ripening can progress into senescence and the flesh can become too soft. The crisp texture of cucumbers and peppers is lost upon exposure to C_2H_4 [16]. The beneficial effects of C_2H_4 are realized by its application to growing plants in the field and orchards, greenhouses, and harvested commodities [16]. Table 13.1 illustrates some of the beneficial effects of ethylene on the quality of fresh fruits and vegetables.

13.2.2 Respiration

As mentioned earlier, fruit and vegetables are living organs of plants that undergo biological and biochemical activity even after they are separated from their plants. The process known as respiration is a sequence of reactions whereby sugars and other substrates, for example organic acids, are oxidized to carbon dioxide, and water vapor and energy are released [27]. The released energy is utilized to synthesize compounds such as proteins and carbohydrates, which together constitute the tissues of the plant. In general, respiration converts the stored energy into usable energy to sustain life. However, harvested or fresh-cut products detached from the plants have a limited energy supply. Basically, the rate of deterioration of harvested products is proportional to their rate of respiration. Hence, the higher the rate of respiration, the shorter the shelf life [28].

The respiration rate of peeled and sliced ripe kiwifruit is doubled compared to the whole fruits, but ripe bananas were unaffected by peeling and slicing [29]. Wound respiration

TABLE 13.1
Beneficial Effects of Ethylene

Benefit	Ethylene Response
Degreening of citrus	Accelerates chlorophyll loss
Ripening of climacteric fruits	*De novo* synthesis of enzymes that promotes ripening
Defense against pathogens	Induces phenylpropanoid metabolism in wounded tissue

in some plant tissues may be related to alpha-oxidation of fatty acids, which oxidizes fatty acids to CO_2, and is responsible for the CO_2 released after slicing of potato tubers [30]. Respiration in plants is an oxidative degradation of sugars, organic acids, and lipids to produce carbon dioxide and water with the release of energy. Modifying the atmosphere around the product by lowering the amount of oxygen with an increase in the amount of carbon dioxide may lower the metabolism with a decrease in CO_2 production and O_2 consumption. The effects of low O_2 and high CO_2 are additive, but the optimal concentrations of the two gases in the storage atmosphere of fruit and vegetables and even between cultivars of the same species may vary [31]. Due to the high affinity for O_2 of the terminal oxidase enzymes in the electron transport chain located in the mitochondria, the amount of O_2 in the surrounding air must be reduced to below 10%. On the other hand, a change to anaerobic respiration will take place if the O_2 concentration approaches 2% [32]. Although high CO_2 and low O_2 levels in the micro-atmosphere of fresh products may extend their shelf life, off-flavor and off-odor developments may be caused by anaerobic respiration, if the oxygen level falls below the 2% critical level [33].

13.2.3 Oxidative Browning

Discoloration occurs at the cut surface of fruits and vegetables as a result of the disruption of compartmentation that occurs when cells are broken, allowing substrates and oxidase enzymes to come in contact with each other [7]. Wounding also induces the synthesis of some enzymes involved in browning reactions or biosynthesis of their substrates [34]. Thus, browning intensity in diverse tissues and crops can be affected by polyphenol oxidase activities and substrate concentrations [33]. Oxidative browning at the cut surface is the limiting factor in storage of many minimally processed fruits and vegetables [7].

Phenylalanineammonialyase (PAL) is a key enzyme in the synthesis of phenolic compounds. The activity of PAL is increased in lettuce midrib tissue with wounding and storage in the presence or absence of ethylene [34]. PAL catalyzes the first reaction in the biosynthesis of plant phenylpropanoid products. The phenolic compounds can then be oxidized by polyphenoloxidase (PPO), thus brown polymers can be produced that can contribute to tissue browning in lettuce [35].

When fruits such as apples and bananas are cut, the cut surfaces usually turn brown within an hour. On the other hand, it takes several hours for the section of cut or shredded vegetables such as lettuce to turn brown. This time lag is considered to be due to the *de novo* biosynthesis of polyphenols [36]. The lettuce tissues with the highest susceptibility to enzymatic browning are the "white" tissue, or the so-called "midribs." This browning is a major problem, which arises during minimal processing and further storage of lettuce midribs [37]. Russet spotting of lettuce is characterized by the appearance of small, reddish-brown spots or lesions on the midribs of the leaves [25].

Ethylene increases the activities of PAL, peroxidase (POD), and PPO. Hence, there is a correlation between PAL activity and development of russet spotting (RS) in ethylene-treated lettuce midribs. Hyodo et al. [38] found a significant increase in some phenolic compounds such as chlorogenic and isochlorogenic acids in the RS-affected tissue. Increased PAL activity promotes the synthesis of cinnamic acid and their derivatives via the shikimic acid pathway. These compounds are then available for lignin synthesis. Ethylene-induced POD activity is correlated with increased lignin formation and cell wall thickening, one of the characteristics of RS. Flavonoids and chlorogenic acid, the other products of the shikimic acid pathway, are oxidized by PPO to form brown compounds [39].

13.2.4 Nutrient Losses: Ascorbic Acid Oxidation

Fruit and vegetables, either processed or "fresh," are major sources of dietary vitamin C for humans. Before consuming, they have to undergo various handling, storage, and processing steps. The vitamin C content of sliced, cut, or bruised fruits and vegetables may diminish rapidly depending on the handling, processing, and storage conditions used [40]. Ascorbic acid is an organic acid found in fruits and vegetables. It is very soluble in water and is sensitive to alkali, oxygen, and the presence of copper, iron, and heat. Ascorbic acid is often considered to be equivalent to vitamin C content, and dehydroascorbic acid (DHAA), the oxidized form of ascorbic acid, also has vitamin C activity [41]. Further oxidation of DHAA converts it to 2,3-diketogulonic acid, which is devoid of biological activity [42]. Figure 13.3 illustrates the oxidation process of ascorbic acid. The loss of ascorbic acid provides a useful index of oxidative deterioration in minimally processed fruits and vegetables [3].

There are two aspects of ascorbic acids degradation. First, ascorbic acid can be oxidized due to mechanical damage as a result of cutting. When the cells of a fresh product are ruptured as occurs during cutting, chemical reactions are initiated that shorten the storage life of the cut product. The enzyme, ascorbic acid oxidase (AAO), released when the cell walls are damaged, will oxidize the ascorbic acid to DHAA, which can undergo further degradation to produce products that no longer possess vitamin C activity [43]. Ascorbic acid can be oxidized due to the physiological activity of AAO that shortens storage life. The auto-oxidation rate of ascorbic acid to DHAA by air is influenced by metal ion concentration and pH. The rate becomes high in alkaline medium, while acetic acid restrains the influence of Fe^{3+} [44]. Albrecht [45] reported that the whole lettuce lost ascorbic acid during storage. Ku and Wills [46] reported that 1-MCP can delay senescence of broccoli. Agar et al. [47] also reported that removal of C_2H_4 from the storage atmosphere increased retention of total ascorbic acid in kiwi fruit. Tay and Perera [48] found that 1-MCP was effective in reducing the loss of ascorbic acid in lettuce.

13.2.5 Water Loss

Plant tissues are in equilibrium with an atmosphere at the same temperature and relative humidity of 99 to 99.5% [7]. Water loss can occur when there is a reduction of water vapor

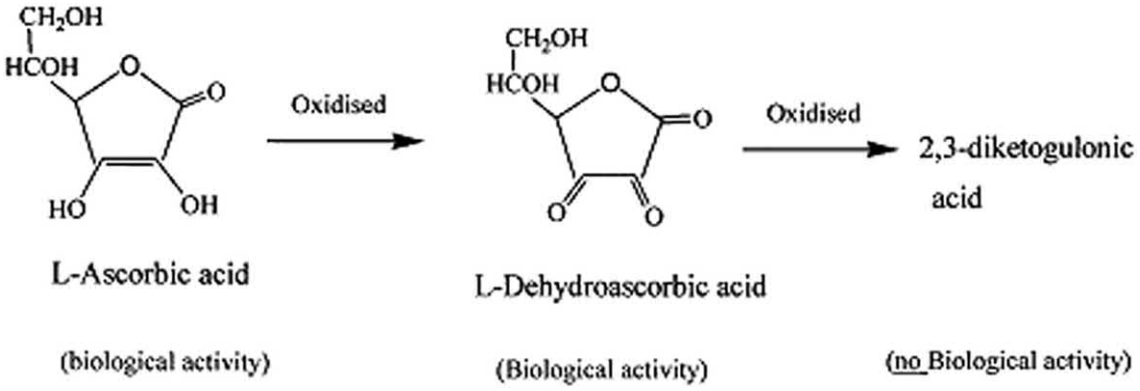

FIGURE 13.3 Oxidation of ascorbic acid.

pressure in the atmosphere surrounding the tissue. In whole fruit or vegetables, water in intercellular spaces is not directly exposed to the outside atmosphere. However, cutting or peeling a fruit or vegetable exposes interior tissues and drastically increases the water evaporation rate [7]. To subsequently maintain the lowest possible water vapor pressure deficit, minimally processed products are routinely handled in packages with a semi-permeable film that has low water vapor transmission rates. Condensation within the package is most severe when the product is at a higher temperature than the storage atmosphere, which is often the case when the product is first placed in the cold-storage room or transport vehicle [7].

13.2.6 Leaf Yellowing in Vegetables

Leaf yellowing is a particularly important quality problem during the transport and storage of fresh or minimally processed green leafy vegetable products. Over the storage period, the leaves could be wrinkled, yellowed, and softened. The decrease in green pigmentation would probably result from the loss of chlorophyll during storage [49]. Tay and Perera [48] found that 1-MCP was effective in retarding storage-induced leaf yellowing in lettuce.

13.3 TECHNIQUES TO EXTEND THE SHELF LIFE

Various approaches are being used to control the undesirable physiological changes that adversely affect the quality of minimally processed products. Refrigeration, humidity control, and dipping in chemical solutions, such as ascorbic acid and calcium, have been used successfully to preserve product quality and enhanced shelf life. Exogenous treatments with calcium chloride ($CaCl_2$) dips were reported to reduce browning and retard flesh softening of vegetables [50]. However, $CaCl_2$ may also cause detectable off-flavors when used at levels higher than 0.5% [51].

Minimally processed products should be refrigerated (0–5°C) to prolong their quality and safety [52]. The removal of C_2H_4 from the storage environment of minimally processed fruits and vegetables can retard tissue softening [52]. Desirable modified atmospheres can be predicted and created within and around commodities by selecting appropriate packaging. Controlled atmospheres can reduce the effects of C_2H_4 on fruit tissues and retard senescence, delay softening, and help to extend the postharvest life [52]. Edible coatings and films are being used successfully with some commodities to provide useful barriers to moisture, O_2, and CO_2 while improving package recyclability [53].

13.3.1 Sanitation

Sanitation is an integral part of minimal processing. Minimally processed fruits and vegetables are essentially damaged tissues. The chances of food pathogens or spoilage organisms' growth in the products are very high. There are three factors that are necessary for foodborne illnesses, namely, the host, pathogen, and exposure [54]. There are no absolute guarantees of the absence of all pathogens in minimally processed products by the current processing methods and technologies. However, a reduction in one or more of the three factors will have a substantial effect in reducing the chances of foodborne illnesses [54]. Considering the growing increase in the consumption of minimally processed and fresh fruit and vegetables in the United States, the FDA sets out clear guidelines to minimize microbial food safety hazards for fresh fruit and vegetables [6]. These guidelines span from "farm to fork" and include Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), and Hazard Analysis Critical Control Points (HACCP).

Sanitization is unlikely to eliminate completely all pathogens on the produce. Therefore, it is important to use sanitizing protocols that are efficient. The efficacy of the sanitizers used to reduce microbial populations is usually dependent upon the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces (cracks, crevices, texture, and hydrophobic tendency), exposure time and concentration of sanitizer, pH, and temperature [55]. Numerous studies have been conducted to evaluate the efficacy and effectiveness of sanitizing treatments on different produce. Researchers have looked into treatments with the use of different sanitizers such as chlorine [55, 56], chlorine dioxide [55–57], hydrogen peroxide [55, 58], and heat [55, 59] as ways to sanitize whole and minimally processed fruits. Most of the research was conducted using fruits such

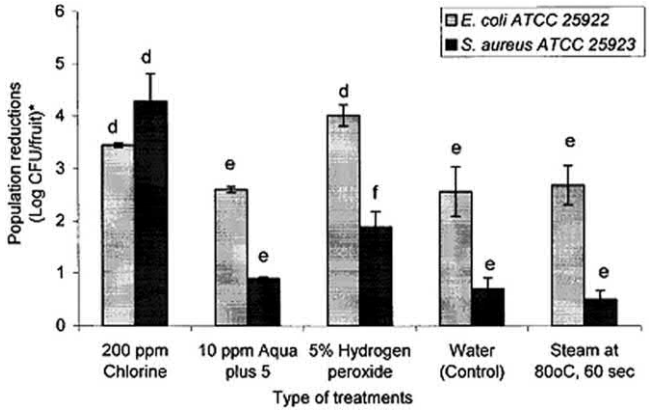

FIGURE 13.4 Comparison of various treatments in reducing inoculated *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 from the surface of star fruit. (From Kiang and Perera [55].)

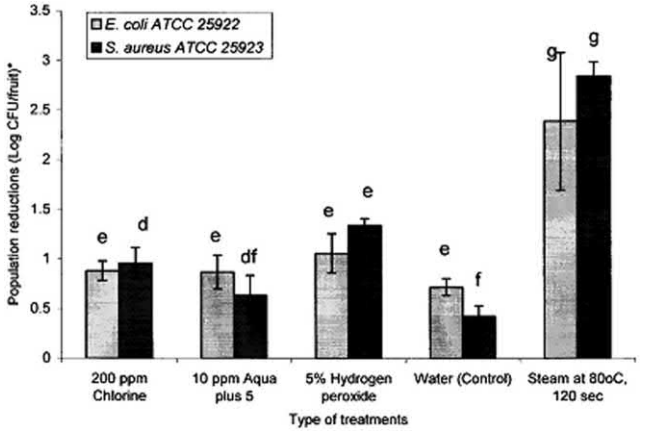

FIGURE 13.6 Comparison of various treatments in reducing inoculated *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 from the surface of pineapples. (From Kiang and Perera [55].)

as apples, oranges, strawberries, and tomatoes, and very little information is available on tropical fruits. Working with tropical fruits, Kiang and Perera [55] showed that the efficacy of sanitization treatments depends on the type of produce and characteristics of produce surfaces. The efficacy of sanitizer treatments on guava, star fruit, and pineapple is shown in Figures 13.4, 13.5, and 13.6. Research has demonstrated that an increase in microbial populations on minimally processed products can have an adverse impact on shelf life: the higher the initial microbial load, the shorter the storage life [56]. While psychotropic Gram-negative rods are the predominant microorganisms on minimally processed products, the primary spoilage organism on prepackaged salads appears to be the fluorescent pectolytic pseudomonads [60].

Washing of fresh fruits and vegetables before cutting is important to control initial microbial load, including mesophilic microflora, lactic acid bacteria, coliform, fecal coliforms, yeasts, molds, and pectolytic microflora [61]. Minimally processed products are generally rinsed in 50–200 ppm chlorine or 5 ppm of chlorine dioxide, which may also aid in reducing the browning reactions [56, 62]. However, product safety, not shelf life, is the critical sanitation issue in minimally processed fruits and vegetables [62].

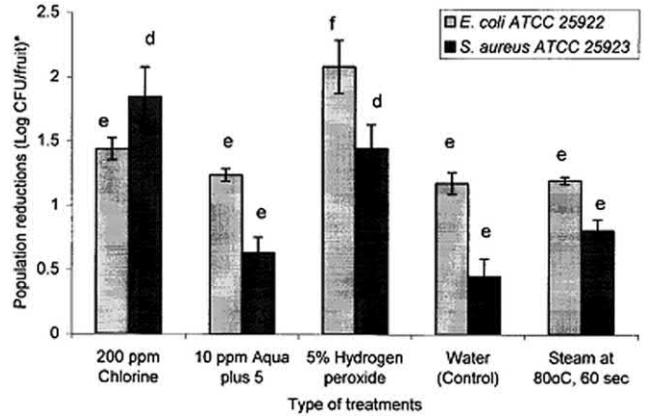

FIGURE 13.5 Comparison of various treatments in reducing inoculated *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 from the surface of guava. (From Kiang and Perera [55].)

13.3.1.1 Chlorine

Chlorine is one of the most widely used sanitizers for the cleaning and washing of minimally processed products. Most processors use a concentrated form of liquid bleach (sodium hypochlorite), although solid calcium hypochlorite or chlorine gas may also be used. However, chlorine gas is difficult to handle, and solid granules of calcium hypochlorite need to be dissolved, which requires additional labor. Free chlorine is an excellent disinfectant; as little as 1 ppm free residual chlorine will sanitize cold process water at a pH of 6.5 to 7.0 [63]. However, it is not effective against *Cryptosporidium* and *Giardia* [64]. Water pH has a significant impact on chlorine activity. At pH values below 5, chlorine gas may be formed and higher levels of free chlorine may cause irritation in workers' lungs and skin. pH values below 6 may cause product discolorations, increase equipment corrosion, and promote the formation of volatile chloramines that may account for health hazards [65]. Also, the chlorination of some organics such as phenols not only results in toxic end products such as chlorophenols but also can impart undesirable tastes and odors to the product treated. However, to date there are no known human health hazards associated with chlorine or chlorine dioxide at the recommended use levels of the two sanitizers [66].

13.3.1.2 Ozone

Ozone has strong oxidizing power and is capable of inactivating microorganisms effectively. To date, there are no indications of adverse human health or environment effects of water ozonization [60, 65]. However, at moderate concentrations, browning reactions may be enhanced in minimally processed products. Ozone finds applications in natural disinfection, water, air purification systems, and fresh fruit and vegetable storage. Recent findings show that ozonized water maintains the quality and flavor of fruits [66]. The off-smell of chlorinated water is absent in ozonized water. (Cl can be detected by humans down to 0.5 ppm, and by some even at 0.25 ppm.)

Ozone is unsurpassed in natural control and killing of common bacteria like *Escherichia coli*, fecal coliforms, molds, and viruses and deactivation of cysts and parasites. It attaches and destroys by oxidation any offending molecule that gets in its path. It rapidly decomposes, leaving no traces of oxidation and does not produce any toxic halogenated compounds.

Chlorine can take minutes or even hours to penetrate the cell walls of bacteria or virus, while activated ozone oxidation occurs within seconds. Unlike chlorine, it does not produce trihalomethanes or chloramines and is non-carcinogenic. There is no evidence of any deaths to humans from over exposure to ozone. Ozone oxidizes organic chemicals into safer elements. During the ozonization process, some compounds like cyanide [67] and ammonia [68] are broken down into nitrogen and water or other safe compounds.

In all reactions, the main by-product after oxidation is O_2. Ozone does not change the pH. Ozonated water is free of algae, bacteria, cysts, mold, viruses, yeast, and parasites. It does not cause an after-taste, and ozonated water is softer with less scale build-up in appliances and plumbing lines. It does not stain plumbing fixtures or cause tub rings. It oxidizes organics, iron, heavy metals, and other contaminates. Water without chemicals or offensive chlorine and sulfur odors is possible with ozone.

13.3.1.3 Electrolyzed Water

Electrolyzed water (EW) has gained popularity as an effective broad-spectrum sanitizer over the last few decades. It has been tested for its bactericidal, fungicidal, and viricidal potential. It is produced by electrolysis of water containing a dissolved electrolyte such as sodium chloride (NaCl). In a water electrolysis plant, the typical cell voltage of 1.8–2.0 V at an operating current density of 100–300 mA cm^{-2} is considerably higher than the equilibrium voltage of 1.23 V [69]. Upon the onset of the electrolysis process, NaCl dissociates into positively and negatively charged ions (Na^+ and Cl^-, respectively). Meanwhile, hydroxide (OH^-) and hydrogen (H^+) ions are also formed in the solution. The negatively charged ions (OH^- and Cl^-) move toward the anode where electrons are released, and hypochlorous acid (HOCl), hypochlorite ion (−OCl), hydrochloric acid (HCl), oxygen gas (O_2), and chlorine gas (Cl_2) are generated. However, positively charged ions (Na^+ and H^+) move toward the cathode where they gain electrons to produce sodium hydroxide (NaOH) and hydrogen gas (H_2) [70]. Two types of EW are generated simultaneously. At the anode, an acidic solution with a pH of 2–3 and oxidation–reduction potential (ORP) >1100 mV and available chlorine concentration (ACC) of 10–90 ppm is produced. This solution is referred to as acidic electrolyzed water (AEW) or electrolyzed oxidizing water (EOW). Meanwhile, at the cathode, a basic solution with a pH 10 to 13 and ORP of –800 to –900 mV is produced, and this solution is termed basic electrolyzed water (BEW). Neutral electrolyzed water (NEW) is produced by mixing the anodic solution with OH− ions or by using a single-cell unit (without diaphragm) from NaCl or HCl [70], whereas slightly acidic electrolyzed water (SAEW) is produced by electrolysis of HCl alone or in combination with NaCl in a single-cell unit without a diaphragm [71].

The main reason for the popularity of EW is the simplicity of its production and application. The acceptance of EW as a sanitizer is evident from its use in a number of applications in various fields including agriculture, medical sterilization, food sanitation, livestock management, and other fields that employ antimicrobial techniques [72]. EW has been used in Japan for several years as an antimicrobial agent. It exhibits antimicrobial activity against a variety of microorganisms. It is active against most common types of viruses, bacteria, fungi, and spores within 5 to 20 sec in food products, food processing surfaces, and non-food surfaces [73, 74]. Various studies have been conducted on the antimicrobial activity of EW on the surfaces of different products including food-handling gloves [74], cutting boards [75], shrimp [76], fish [77], beef [78], pork [79], poultry carcasses [77], fruits [80], and vegetables [81]. Acidic electrolyzed water (AEW) is effective against Listeria even at refrigerated temperatures. AEW and Cl exhibit the same bactericidal effect against Listeria with a 1.2 and 1.6 $\log_{10}$ CFU/g reduction after 5 seconds as compared to the number recovered from unwashed peaches [80]. Recently Rahman and others [82] reviewed the current trends and future prospects of electrolyzed water as a novel sanitizer in the food industry. They concluded that EW is a sustainable green concept and has several advantages over traditional cleaning systems, including cost-effectiveness, ease of application, on-the-spot production, and safety to human beings and the environment.

13.3.1.4 Chlorine Dioxide

Chlorine dioxide-based sanitizer has strong oxidizing properties and is efficient at killing microorganisms. Due to its true oxidizing characteristics, there is no residue to affect the quality of the food. It is non-toxic, low-corrosive, and pH-independent. Unlike chlorine, it does not combine with organic compounds to form toxic chlorinated compounds and does not react with ammonia. The product formulation is comprised of biodegradable compounds, which break down after use to form natural substances. It is approved for food use, by the US FDA, USDA, and Environmental Protection Agency (EPA) [64]. The killing power of a sanitizer is expressed as the product of the concentration C (mg/L) and time t (min) of exposure of the sanitizer to the microorganisms concerned. Table 13.2 illustrates the relative killing power (Ct) of chlorine dioxide, chlorine, and ozone on the common water-borne microorganisms, *Giardia*, *Cryptosporidium*, and viruses [64].

13.3.2 1-Methylcyclopropene (1-MCP)

The chemical 1-methylcyclopropene (1-MCP) is currently formulated as SmartFresh™, a powder having 3.8% of the active ingredient for postharvest use in fruits and vegetables and EthylBloc™ for the use in flowers. SmartFresh™ liberates the active 1-MCP when added to water and is stable in its powder form. The chemical structure of 1-MCP is shown in Figure 13.7. Acute toxicity, mutagenicity, and product chemistry studies conducted on the SmartFresh™ formulation indicate that the toxicology profile is acceptable. In addition, 1-MCP has a

TABLE 13.2
Required Ct Values (mg min/L) for Inactivation of Pathogenic Microorganism in Water

	Inactivation Level (Log)	ClO_2	Cl_2	O_3
Cryptosporidium	0.5	138	N/A	4.9
Cryptosporidium	3	830	N/A	30
Giardia	0.5	4	17	0.23
Giardia	3	23	104	1.43
Virus	2	4.2	3	0.5
Virus	4	25.1	6	1.0

Source: EPA [64].

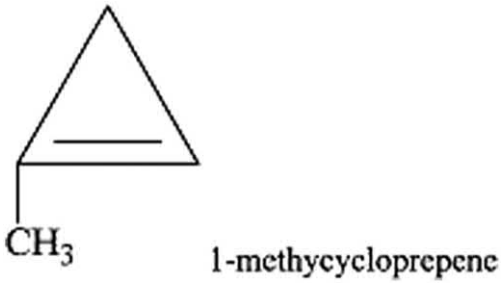

FIGURE 13.7 Structure of 1-MCP.

non-toxic mode of action, is applied at extremely low parts per billion dose levels, and has no exception of measurable residues in food commodities. The EPA has classified 1-MCP as a plant regulator structurally related to plant-containing materials. Watkins [83] recently reviewed the status of 1-MCP and stated that EPA registration was granted for flowers in April 1999, and in 2002 it was approved for use in apples. It is currently approved for use in over 42 countries, and according to Agrofresh, the manufacturers of SmartFresh™, it also provides better firmness maintenance for highly ethylene-sensitive fruits like plums and persimmons [84]. In 2015, EPA [85] approved a new 1-MCP technology for use in fruits.

1-MCP is a cyclic olefin, analogous to the photodecomposition product of diazocyclopentadiene (DACP), and, to date, is the most useful compound among recently developed inhibitors of ethylene response. 1-MCP is a gas at room temperature, has no obvious odor at required concentration levels, and is non-toxic. It is relatively stable in dilute gas phase for several months [86], but is unstable in the liquid phase, polymerizing even at low refrigerator temperatures [87].

Autocatalytic ethylene production requires up-regulation, by ethylene, of ACC synthase and ACC oxidase activity [88]. Lelievre et al. [89] demonstrated that treatment with 1-MCP resulted in reduced accumulation of ACC synthase/oxidase transcripts and ethylene production in pears, during chilling. Presumably, 1-MCP competitively binds to a metal in the ethylene receptors involved in feedback regulation and consequently blocks the ability of ethylene to up-regulate. However, in the case of wounding whereby ethylene production is not autocatalytic, another mechanism must operate in order for 1-MCP to reduce ethylene synthesis. 1-MCP is capable of allene-type arrangement, which probably is a crucial factor when binding occurs [90]. The affinity of 1-MCP to the receptor is about ten times that of ethylene [91]. Once 1-MCP is bound to the ethylene receptor, ethylene binding is impossible and therefore fails to elicit its subsequent actions on the tissue.

1-MCP provides protection for a longer period of time than other potential ethylene inhibitor compounds [92]. It acts at concentrations of 0.5 nLL^{-1}, and the effect is prolonged. 1-MCP has been reported to delay or reduce ethylene-induced effects on senescence in a variety of potted flowering plants and cut flowers [93–95]. The effects of 1-MCP on fruits and vegetables include inhibiting the ripening of tomatoes [96], delaying senescence of eggplant [97] and broccoli [98], inhibiting the yellowing of cut lettuce [48], extending the firmness and shelf life of minimally processed apples [99], and degreening of mandarin oranges, while not suppressing other ethylene-induced effects such as chilling injury [100]. Also, after treatment, tomatoes, bananas, and other plant materials were found to have no response even to very high amounts of ethylene [87].

1-MCP reduces ethylene synthesis and respiration rate and thus decreases the activities of phenylalanineammonialyase (PAL) [101]. PAL is a key enzyme in phenolic synthesis. The phenolic compounds can then be oxidized by polyphenoloxidase (PPO), producing brown polymers that can contribute to tissue browning in lettuce [26]. Fan and Mattheis [102] reported that short-term 1-MCP treatment prevents the development of RS and isocoumarin accumulation in iceberg lettuce. The russet spotting (RS) level was also lowered in 1-MCP-treated lettuce [48]. This indicates that ethylene is important for the development of RS in lettuce.

13.3.3 Edible Coating

Coatings of fruits and vegetables have been known for many years [103]. Edible coatings are generally made from one or more of four major types of materials: lipids, resins, polysaccharides, and proteins [104]. Coatings made from polysaccharides (cellulose, pectin, starch, alginates, chitosan, carrageenan, gums) are generally good barriers and adhere well to cut surfaces of fruits or vegetables, but their hydrophilic nature makes them poor barriers to moisture [48, 105].

Almost all minimally processed fruits have all or part of the peel or outer protective coating removed. This allows the entrance of spoilage organisms and dehydration of the fruit's tissues. Dehydration may be partially responsible for some of the softening that is observed in minimally processed fruit products. The use of edible coating or plastic packaging is necessary to retard moisture loss by providing a barrier to water vapor resulting in a high relative humidity environment, as well as to minimize microbial contamination [105].

Attempts have been made to control leakage from minimally processed (peeled) grapefruit segments with an edible coating of calcium alginate, with varying levels of coating firmness. The coatings effectively enhanced the firmness

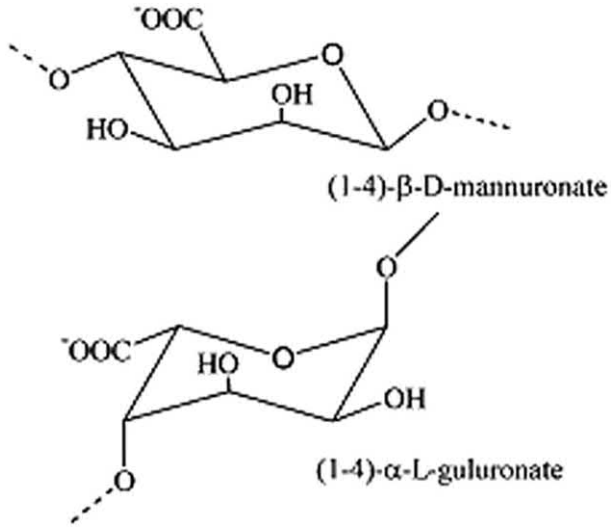

FIGURE 13.8 The alginate monomer units.

of segments, but the effects on fluid loss were negligible. An 89% increase in tissue firmness was accompanied by only a 16% decrease in fluid loss [106]. Alginate is a group of naturally occurring polysaccharide (MW ~240,000) isolated from brown seaweed composed of D-mannuronic acid and L-guluronic acid subunits [107]. Figure 13.8 shows the alginate monomer unit. It is a non-toxic and biodegradable polymer that can be used in food [108]. Sodium alginate and polypropylene glycol alginate are commonly used as thickeners in foods such as ice cream and fruit-filled snacks [109]. Alginates form gels with a number of divalent cations [110]. For food purposes, calcium is particularly suitable because of its non-toxicity. Figure 13.9 illustrates water-soluble sodium alginate polymer cross-linked with Ca^{2+} (long chains are interconnected) to form flexible, translucent gels. The longer the calcium alginate polymer is in contact with the calcium chloride solution, the more rigid the gel will become [107].

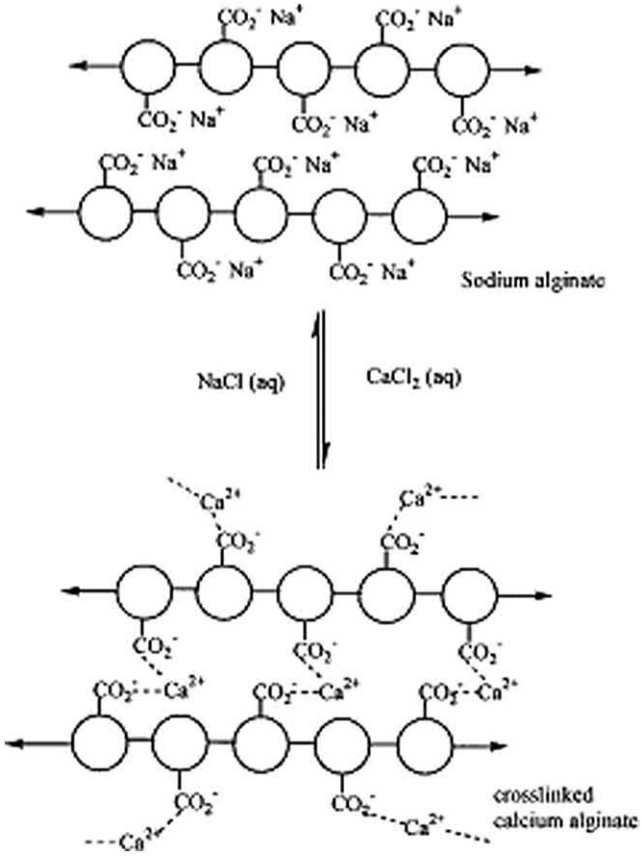

FIGURE 13.9 Alginate polymer in NaCl or $CaCl_2$ solution. (From Perera et al. [112].)

13.3.4 Firming Agents

Treatments with $CaCl_2$ dip have been reported to reduced browning and flesh softening of vegetables [50]. Tay and Perera [48] found that the calcium alginate-coated lettuce had pronounced improvement in crispiness. The thin calcium alginate edible coating retards moisture loss and prevents dehydration that may be partially responsible for some of the crispiness. However, the calcium alginate-coated lettuces were more likely to suffer bruises during the coating process. Hence this may be the reason why the reduction in browning was not so significant. Izumi and Watada [50] reported that exogenous treatments with calcium chloride ($CaCl_2$) dip reduced browning and retarded flesh softening of vegetables. The alginate-coated lettuces were dipped in $CaCl_2$ solution for a cross-linking reaction. Hence the $CaCl_2$ could also be responsible for the crispness. Dipping times ranging from 1 to 5 min have been used in most of the published work [111]. Different calcium salts have been studied for decay prevention, sanitation, and enhancement of the texture of minimally processed fruits and vegetables. Calcium lactate, calcium chloride, calcium phosphate, calcium propionate, and calcium gluconate are often used when the objective is the preservation and/or the enhancement of the product firmness. The selection of the appropriate source depends on several factors. Solubility, flavor change, and interaction with food ingredients are the most significant ones. The concentrations of the calcium salts used usually ranged from 0.5 to 3%, and dipping time ranged from 1 to 5 minutes as mentioned in most of the published work [111].

13.3.5 Intelligent and Active Packaging

Minimally processed fruit and vegetables are commonly packed in perforated plastic boxes or plastic bags. In addition, active packaging technologies can also be incorporated. Active packaging technologies include some physical, chemical, or biological action, which changes the interactions between the package, product, and headspace of the package in order to achieve the desired outcome [113]. The most common active packaging systems act to scavenge oxygen from the package, and they may even be activated by an outside source such as UV light [114]. Active packaging is typically found in two types of systems: sachets and pads which are placed inside the packages, and active ingredients that are

incorporated directly into the packaging materials. Sachets were developed in Japan in the late 1970s for scavenging oxygen from the headspace of packages. The principle is essentially the process of rusting, where ferrous oxide is oxidized to ferric oxide in the presence of KCl used as a catalyst. Similarly, a carbon dioxide absorber was made from hydrated lime [$Ca(OH)_2$]. When carbon dioxide comes in contact with this material, it forms a precipitate of calcium carbonate, and the CO_2 in the headspace is reduced.

Small amounts of ethylene can trigger many chemical reactions and impart ripening and senescence in fruit and vegetables. A number of catalytic oxidizers have been combined with adsorbents to remove ethylene from air. Examples include potassium dichromate, $KMnO_4$, iodine pentoxide, and silver nitrate, each respectively on silica gel. A novel palladium-promoted material with a significant ethylene adsorption capacity at room temperature has recently been patented [115]. Catalytic oxidation of ethylene by $KMnO_4$ is one of the most commonly used ethylene-scavenging processes. In one system known as "BioSwitch" [116], an antimicrobial agent is released on command when bacterial growth occurs. The basic concept is that when a change in the environment such as pH, temperature, or UV light occurs, the antimicrobial responds accordingly.

13.4 HARVESTING AND HANDLING OPERATIONS

Harvesting and handling are important operations in extending the shelf life of minimally processed fruit and vegetables. Major factors involved include (i) careful harvesting so as not to injure the product, (ii) harvesting at optimal horticultural maturity for intended use, (ii) rapid removal of "field heat," (iv) sharp cutting methods to cause minimal cell disruption, (v) maintenance of low temperature throughout, and (vi) good sanitation practices. When these are practiced, the implementation of optimum storage conditions through modified atmospheres can be quite effective at maximizing the shelf life and quality of the product.

Harvesting should be done in such a way as to minimize any bruising or injuring of the tissues of fruits and vegetables, which might raise the respiration rate and, thus, reduce the shelf life. A number of studies have shown that a drop as small as 10 cm could cause bruising injuries in some fruits [117–119]. The maturity of the fruit or the vegetable at harvest is very important. As a general rule of thumb, vegetables are harvested when they are tender, that is, in their early growth period when lignification of the fibers has not occurred, e.g. carrots, beans, peas, and asparagus. At this stage of maturity, most of the common vegetables contain the highest amount of soluble sugars, and these are tender, sweet, and flavorful and contain the maximum amount of nutrients, such as vitamins and minerals.

On the other hand, fruits are harvested when they are fully mature, but unripe. At this stage of maturity, they carry the maximum amount of nutrients and develop the flavor, color, and texture when they undergo ripening with which all of us are familiar. If harvested at the ripe stage, the fruits will be soft and will be difficult to handle and transport. Therefore, the optimum stage of maturity of harvest is required for each fruit and vegetable. Once harvested, the produce needs to be cooled as rapidly as possible to remove the "field heat." Otherwise, the temperature within the stack of fruit or vegetables can increase the rate of respiration and cause the produce to deteriorate rapidly. Field heat can be removed by one of several methods. Hydro-cooling by immersion in cold water is one method of cooling rapidly, but it may not be suitable for certain types of fruits and vegetables. Certain fruits (e.g. golden delicious apple) that have tiny passages opened to the interior of the fruit from the calyx end provide a pathway from outside to the interior of the seed cavity. Fruits washed by the immersion method could get infected when contaminated water gets inside the fruit through these passageways [120]. In order to avoid such undesirable happenings, the wash water should be at a higher temperature than the fruit. Thus, good sanitary practices are important when handling fresh fruit and vegetables for minimal processing.

Air cooling is probably the ideal, as it does not lend itself to possible contamination as discussed above. In air cooling, it is important to maintain a high level of relative humidity of the cooling air. Otherwise, it may lead to desiccation of the product and a possible economic loss to the producer. Another important aspect is to choose the right varieties of produce for minimal processing. For example, certain apple varieties have a higher tendency to brown (browning potential) than others [121, 122]. Therefore, those varieties that have lower browning potential are used to minimize browning changes during processing and storage.

The sharpness of cutting blades significantly affects the shelf life of minimally processed products. Dull knives could damage and bruise several layers into the product tissues and could cause extensive cell damage. Consequent physiological and chemical changes could result in loss of texture, color, and susceptibility to the growth of microorganisms [52]. The maintenance of low temperatures is another critical factor in extending the shelf life of minimally processed products. The fruit and vegetable tissues that have been peeled, cut, sliced, or grated essentially contain damaged cells, which cause elevated respiration rates. Therefore, it is most essential that the cut products are chilled at temperatures as low as possible to reduce the rate of respiration and control biochemical and microbial activity.

It is well-known that the shelf-life of some tropical and sub-tropical fruits and vegetables can be limited by chilling injury, a disorder induced by low (0–4°C) but non-freezing temperatures. The extent of chilling injury is influenced by temperature, duration of exposure to a given temperature, and chilling sensitivity of the particular fruit or vegetable. The symptoms of chilling injury may not be evident while produce is held at chilling temperatures, but they become apparent after transfer to higher temperatures [123]. The susceptibility to chilling injury in tropical fruits may only manifest in whole or unprocessed products as has been observed during the storage of pineapple at 8°C [124], papaya at 10°C [125], durian at 15°C [5, 126], and jack fruit at 10°C [127]. However, there was

no chilling injury reported in minimally processed pineapple [128] and durian [126] during storage at 2°C and papaya and melon stored at 4°C [129].

13.5 FOOD SAFETY REGULATIONS

The natural protection offered by the peel/skin of the fruit is generally lost due to peeling, cutting, and slicing operations on minimally processed products. Hence, the product becomes highly susceptible to microbial spoilage [130]. If the sanitation of the whole fruit is not properly carried out, cross-contamination may occur during the minimal processing operations. Leakage of cell sap from the damaged tissues allows the growth of some species of yeasts such as *Saccharomyces cerevisiae* and *Saccharomyces exiguous* [131]. The damaged plant tissues increase their susceptibility to contamination with human pathogens.

Regulatory initiatives specific to minimally processed products are still under development in many countries, although the United States and most European countries already have relevant regulations. Most of them limit the counts of aerobic microorganisms to 10^6 CFU/g at the expiry date of the product. In addition, pathogenic microorganisms are not permitted or are greatly restricted (*E. coli*, *Listeria monocytogenes*) in ready-to-eat meals prepared from raw vegetable products [130].

13.5.1 Regulatory Requirements

The current regulatory requirements for minimally processed produce and Current Good Manufacturing Practice requirements for foods (CGMPs) are stipulated under Code of Federal Regulations Title 21 Part 110 [132]. These regulations establish CGMPs in manufacturing, packing, and holding human food. However, "raw agricultural commodities (RACs), as defined in section 201(r) of the Federal Food, Drug, and Cosmetic Act (the Act), are not subject to the CGMP requirements by virtue of the exclusion in 21 CFR 110.19. Section 201(r) defines a raw agricultural commodity as any food "in its raw or natural state." Fresh-cut fruits and vegetables are not RACs because they are no longer "in [their] raw or natural state" and instead have become "processed food" as that term is defined in the Act. Section 201(gg) of the Act defines a "processed food" as "any food other than a raw agricultural commodity and includes any raw agricultural commodity that has been subject to processing, such as canning, cooking, freezing, dehydrating, or milling." Under 21 CFR 110.3, the definitions in section 201 of the Act apply to Part 110. Thus, fresh-cut fruits and vegetables are appropriately considered "processed foods" and are subject to the CGMPs in Part 110. The conclusion that fresh-cut produce are not RACs is consistent with the preamble to the proposed revisions to the CGMP regulation (44 FR 33238 at 33239, June 8, 1979), which states, when discussing the exclusion for RACs, that such products may be excluded because "food from those commodities is … brought into compliance with the Act at the later stages of manufacturing, processing, packing, or holding." The CGMPs establish food safety practices applicable to processors who manufacture, process, pack, or hold processed food. The FDA believes that the recommendations in this guidance complement the CGMPs by suggesting "more specific food safety practices for processors of fresh-cut produce" [133].

Guidelines for microbial examination of ready-to-eat foods are given by Food Standards Australia and New Zealand [134]. The standard plate count (SPC), also referred to as the aerobic plate count or the total viable count, is one of the most common tests applied to indicate the microbiological quality of food. The significance of SPC, however, varies markedly according to the type of food product and the processing it has received. SPC is not applicable to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods, and foods incorporating these (such as sandwiches and filled rolls). It would be expected that these foods would have an inherent high plate count because of the normal microbial flora present. For minimally processed products *Escherichia coli* should be less than 3 CFU/g and pathogenic strains of *E. coli* should be absent. In addition, the limits for the following pathogens are: Coagulase +ve staphylococci should be less than 10^2 CFU/g, *Clostridium perfringens* less than 10^2 CFU/g, *Bacillus cereus* and other pathogenic *Bacillus* spp. less than 10^2 CFU/g, *Vibrio parahaemolyticus* less than 3 CFU/g, *Campylobacter* spp. not detected in 25g, *Salmonella* spp. not detected in 25g, and *L. monocytogenes* not detected in 25g [134].

In New Zealand, the latest Food Act has been passed to cover minimally processed food products. From 1 March 2016, there is a mandatory requirement for processors of minimally processed products to implement a Food Control Plan (FCP) and a National Program 1 [135]. If it is a new business, then they need to implement these requirements now, but if it is a continuing business, then they have until 31 March 2017 to implement them.

13.6 CONCLUSION

Minimal processing is a growing processing trend, which offers consumer convenience, "freshness" of quality, nutrition, and safety. Because it involves removing or reducing the natural barriers to respiration and deterioration, it offers scientists an enormous challenge in trying to extend the shelf life of minimally processed fresh produce. However, the consumer demand for minimally processed products and changes in the perceptions of consumers of the "freshness" of quality of fresh produce and the convenience of such products warrant further research and developments in this area. In order to do this, a deeper understanding of the physiology and biochemistry of the plant materials used for minimal processing is essential. An outcome of such understanding is the development of 1-MCP as an ethylene block. While this may offer some hope of extending the shelf life of minimally processed fresh produce, more research is needed for its application to different fruits and vegetables and their diverse cultivars. In addition to technological methods for extending the shelf life of minimally processed products, there are regulatory

requirements that cover the safety of these products in many countries, which offer safeguards for the safety of these products to the consumer.

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14 Preservation of Part-Baked Products

Mehmet Murat Karaoglu

CONTENTS

14.1 INTRODUCTION

Bakery products are the most widely consumed foods in the world and are gaining popularity as processed foods because of their availability, convenience (i.e. they are ready to eat with a simple process after purchase), comparatively good shelf life, and sensory acceptance [1, 2]. Baked products are generally consumed as fresh and have a short shelf life since undesired changes begin just after the baking process. The major undesirable changes are (i) a decrease in humidity, (ii) moisture redistribution, (iii) starch retrogradation, (iv) increased firmness, and (v) loss of aroma and flavor. Moreover, these changes cause bakery products to go stale, which is the main reason for consumer rejection of bakery goods [2, 3].

Bakery products, especially those with high moisture content, are mostly cellular solid food systems, which consist primarily of gluten and starch fractions [4]. During and after baking, structural changes occur particularly in starch and protein and cause a significant effect in the consumption quality of the product. Starch is the main component of most bakery products, and its gelatinization induces major structural changes during baking, especially in bakery products of high moisture content. The swollen starch granules and partially solubilized starch act as important structural elements of baked products. The other important components are gliadins and glutenins, which are transformed into a cohesive viscoelastic gluten protein network. The viscoelastic protein network in the fermenting dough plays a major role

in retaining the carbon dioxide produced during the process. These gas retention properties in turn determine the volume and crumb structure of the resulting products. On heating, the gluten transforms from a gel to a coagel by polymerization. Consequently, the change from dough to final product involves changes in both the starch and protein fractions. At the macroscopic level, baking induces the solidification of dough and a change from a very viscous liquid (foam structure) system with gas cells to a semi-rigid elastic solid (elastic crumb sponge) with an open pore system [5–8]. On cooling and aging of the product, staling comes into effect, which significantly affects the quality of bakery products [9].

Water activity (a_w) can be used as an indicator of the stability of foods because it influences the microbial growth, spoilage rates, and physical/chemical properties. As the water activity increases, the ability of water to act as a solvent, medium, and reactant increases. The water activity also plays a role in the textural properties of food, because the physical state of water in a food system significantly affects its structure, physical, and chemical properties. Foods with high water activity generally have a texture defined as moist, juicy, tender, and chewy. However, when foods' water activity is lowered, their texture changes and is normally described as hard, stale, dry, and crunchy. When looking at low moisture-content foods, they have a texture described as crispy and crunchy. Thus, when their water activities are increased, the texture generally changes to soggy [10].

The pH and water activity of bakery products can be classified into different groups for a better approach to understanding storage stability (Table 14.1). These groups are high-acidic (pH < 4.6), low-acidic (pH > 4.6 and pH < 7), and non-acidic bakery products (pH > 7), and low-moisture (a_w < 0.6), intermediate-moisture (a_w between 0.6 and 0.85), and high-moisture bakery products (a_w > 0.85 and generally between 0.95 and 0.99). The classification of products according to their pH and a_w is helpful in recognizing the spoilage potential and safety risk of bakery products [2, 11].

The rate of spoilage in baked products depends on the water activity of the product. Water activity is the most important factor influencing microbiological activity in bakery products [12]. The minimum a_w values for the growth of spoilage microorganisms in some baked products are shown in Figure 14.1. For low-moisture baked products (a_w < 0.6), microbiological spoilage is not a challenge. Osmophilic yeasts and molds are the predominant spoilage microorganisms in intermediate moisture products (a_w 0.6–0.85). In bakery products with high moisture (a_w 0.94–0.99), almost all bacteria, yeasts, and molds are capable of growing [2, 13].

Storage temperature, relative humidity, packaging material and the gaseous environment surrounding the product, level of preservatives, pH, and especially the moisture content and a_w significantly affect the spoilage problems in baked products. Food spoilage is a change in a food that makes it unacceptable or undesirable for consumption. The spoilage problems of bakery products can be subdivided into three main groups: (i) physical spoilage (moisture loss, staling), (ii) chemical spoilage (rancidity), and (iii) microbiological spoilage (yeast, mold, bacterial growth). The shelf life of low- and intermediate-moisture bakery products is limited by physical

TABLE 14.1
pH and Water Activity (a_w) Range of Bakery Products

Product	pH Range	Product	a_w
High acidic		Low moisture content	
Sourdough bread	4.2–4.6	Cookies	0.2–0.3
Low acidic		Intermediate moisture content	
Cake, angel food	5.2–5.6	Fruit cake	0.73–0.83
White bread	5.3–5.8	Cream-filled cake	0.78–0.81
Whole wheat bread	5.6	Danish pastries	0.82–0.83
Date nut bread	6.1–6.7	Chocolate-coated doughnuts	0.82–0.83
Nonacidic		High moisture content	
Crumpets	6–8	Baked cake	0.90–0.94
Cake, pound	6.6–7.1	Fruit pies	0.95–0.98
Cake chocolate	7.2–7.6	Bread	0.95–0.98
Carrot muffin	8.7	Pizza	0.99

Source: Adapted from Smith et al. [2]; Schmidt and Fontana [13]; McGlynn [110].

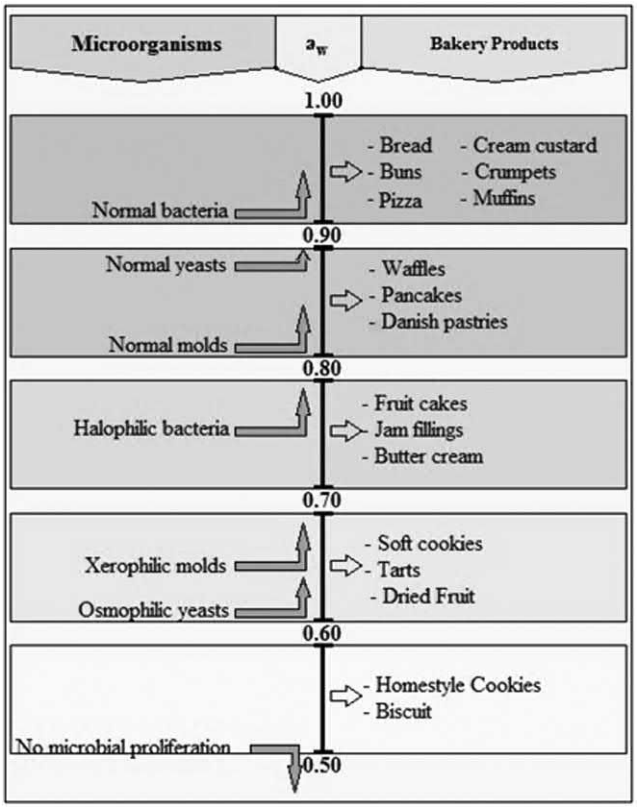

FIGURE 14.1 Minimum water activity (a_w) values for growth of spoilage microorganisms in some bakery products. (Adapted from Smith et al. [2]; Schmidt and Fontana [13].)

and chemical spoilage processes, while microbiological spoilage is the main concern for intermediate- and high-moisture products. The vast majority of partially baked (i.e. part-baked) products are high-moisture and low-acid bakery products, thus ideal substrates for microbial development [2, 11].

14.2 STALING AND SPOILAGE IN BAKED PRODUCTS

14.2.1 Microbial Spoilage

Soft bakery products are dynamic systems that undergo physical, chemical, and microbiological changes during processing and storage, and this limits their shelf life. Physical and chemical changes result in the loss of freshness, in terms of desired texture and taste, and lead to the progressive firming of the crumb. Microbiological spoilage by bacteria, yeasts, and molds consists of visible mold growth, invisible production of mycotoxins, and formation of off-flavors [14]. From these factors, the most important one limiting the shelf life of the product is microbial spoilage [15].

Besides going stale, microbial spoilage by bacteria, and especially molds, remains responsible for enormous economic losses in the bakery industries [14, 16]. It is considered that about 60% of the spoilage problems are caused by molds, resulting in losses of 1 to 5% of the total bread production. Furthermore, there is evidence that molds produce mycotoxins, which can have an adverse effect on human health. Viable vegetative molds and mold spores are normally inactivated by the heat during the baking process due to thermal shock. However, post-baking contamination occurs during the cooling and packaging stages. In addition, mold, bacterial, and yeast spoilage can also occur in bakery products. In particular, ropiness caused by *Bacillus subtilis* is the most common bacterial spoilage in bakery products such as bread [15, 17]. Heat-resistant spores of *Bacillus* species are not inactivated during baking and can then germinate and grow within 36–48 h inside the loaf to form a characteristic soft, stringy, brown mass with an odor of ripe pineapple or melon due to the release of volatile compounds, such as diacetyl, acetoin, acetaldehyde, and isovaler-aldehyde. Their growth can cause undesirable texture or flavor changes and can spoil the product. The crucial step during the part-baking process with regard to the propagation of bacilli spores is the re-baking or final baking process [18]. Yeast spoilage is the least common of all types of microbial spoilage since yeasts do not survive in the baking conditions. Contamination rather occurs during the cooling stage of products, and, in the case of industrial bread, it happens especially during the slicing step [14, 19].

The prevention of post-baking contamination can be achieved by (i) placing under aseptic conditions immediately after baking, (ii) destruction of post-baking contaminants on the surface of products after packaging, and (iii) controlling the growth of post-baking contaminants in the packaged products. These preventions of contaminations are the basic strategies to extend the microbiological shelf life of bakery products. The mold-free shelf life of many bakery products could be increased if post-cooking contamination can be prevented by packaging as soon as possible after baking. However, the success of preventing post-baking contamination of bakery products has achieved limited success. For this reason, attention has focused on the other methods, such as ultraviolet light, infrared irradiation, microwave heating, low-dose γ-irradiation, pulsed light, and high pressure to control any post-processing contamination of mold spores [11, 14, 15, 20]. However, the most practical, efficient approach to extend the microbiological shelf life of bakery products is to control the growth of any post-baking contamination in the packaged products. Post-baking contamination can be controlled by reducing a_w of reformulated product, chemical preservatives (such as sorbates or propionates), and modified-atmosphere packaging [2].

14.2.2 Staling

In spite of all this, the most important factor affecting the consumption quality of bakery products is staling [21]. The undesirable changes occur in bakery products, especially soft products, such as bread, cake, and doughnuts. The decrease of crust crispiness or toughening of the crust, firming of the crumb, and loss of flavor over storage time are known as staling. The staling phenomenon can be explained by two basic mechanisms: (i) the moisture migration from the crumb to the crust during storage leaving the crust soft and leathery and the crumb hard and dry, and (ii) recrystallization of starch that starts when the baked product is being cooled down after baking [10, 22, 23]. However, crumb hardening is a complicated process resulting from events that happen at the same time.

Since starch is the major constituent in the bakery product, the physical changes accompanying the retrogradation of starch have been suggested as the main reason for staling. Starch retrogradation is a complex process in which gelatinized starch molecules re-associate to form double-helical crystalline structures. Therefore, structural changes in starch occurring during baking and storage have an extremely important effect on the structure, texture, and quality of the baked products [10, 24]. Depending on the source, starch consists of 20–30% amylose, an unbranched α-1-4 polymer of D-glucose, and 70–80% amylopectin, which consists of α-1-4 links with α-1-6 links every 25–30 glucose residues. The recrystallization tendencies of amylose and amylopectin differ from each other [25]. Amylose recrystallizes rapidly upon cooling, and recrystallized amylose can be partially responsible for the initial crumb firmness and in the early stages of aging [26]. On the other hand, the recrystallization of the amylopectin fraction within and between the swollen and deformed gelatinized starch granules occurs over a longer period (i.e. several days after baking), probably because of the branched structure of amylopectin. For this reason, it determines the long-term development of structure and crystallinity in starch systems like bakery products. It is more often referred to as retrogradation [27–29]. Amylopectin retrogradation, in particular the formation of double-helical structures and crystalline regions, is mainly responsible for crumb firming. The optimum

temperature for the retrogradation of amylopectin occurs between glass transition and ice-melting temperatures [30].

At present, many reports have shown that starch retrogradation is not the only factor in the staling of bakery products. Water also plays a critical role in bread staling. When the recrystallization of starch occurs, water molecules are incorporated into the crystallites and the distribution of water is shifted from gluten to starch, thereby changing the nature of the gluten network. Besides the molecular arrangement of starch, water has also an important role in crumb firmness due to its plasticizing effect [22]. Other factors affecting crumb firmness are amylose recrystallization, moisture loss, interactions between starch and gluten, and moisture redistribution [9]. In the latter case, gluten, a continuous phase in the bread, is cross-linked by the gelatinized starch resulting in increased crumb firmness [23].

14.3 RETARDING STALING OF BAKED PRODUCTS

The aging of baked products has attracted research interest for many years. Researchers and the baking industry have attempted to retard staling by applying different methods. Many efforts have been focused on the development of different additives such as emulsifiers, hydrocolloids, and enzymes such as α-amylases, hemicelluloses, lipases, and reformulation with these additives. These could extend the shelf life of the soft bakery products by retarding the staling process during storage. In addition to these additives, other different approaches, such as fermentation, baking, and chilling conditions, freezing, and appropriate storage conditions have been studied [31–33]. Recently, part-baking has been used to extend the shelf life of bakery products and to offer fresh-like baked products after final baking before consumption.

There are three important methods used to extend the shelf life of bakery products. These are (i) frozen dough technology, (ii) freezing of bakery products, and (iii) production of partially baked (i.e. part-bread) products. Although freezing is appropriate for extending the shelf life of bakery products, the quality of frozen products can be affected in terms of texture and aroma as compared to fresh ones [33, 34]. The freezing process can be applied to dough in order to interrupt the fermentation process for bakery products before proofing. The frozen dough led to bakery products of reduced volume, mainly due to the physical damage caused to the protein network structure and the deterioration of yeast during frozen storage. Thus, the market is moving from frozen dough to part-baked products [35, 36]. These difficulties can be minimized by using strong wheat flour and freeze-tolerant yeasts. However, the baking process of frozen dough still needs specific flour quality, freezing and thawing conditions, and dough handling. In the part baking process, the product is ready for consumption after a simple cooking process [31]. In view of certain difficulties related to the preservation of yeast activity in frozen dough, a better way of maintaining freshness could consist of freezing part-baked products [37]. The freezing process can also be applied to part-baked products, and successful results could be obtained. The freezing of part-baked fermented bakery product is an easy way to prolong the shelf life and keep its freshness [31].

14.4 PART-BAKING PROCESS

14.4.1 Understanding Part-Baked Products

Changing consumer habits and new social lifestyles have had an important impact on the bakery industry. In recent years, there has been a growing demand for high-quality foods based on convenience with minimum requirements for preparation time. Part-baked products have an advantage in relation to fully baked ones since they need very little preparation time in the oven [38]. Another advantage of the part-baking process is its limited microbial activity and chemical reactions due to pre-baking; thus it enables a longer shelf life [39]. In addition, recent consumer trends have promoted an increase in the consumption of partially baked bakery products due to the availability of fresh products at any time of the day [9].

The baking of bakery products is a complex heating process that causes a series of physical, chemical, and biochemical changes, such as volume expansion, evaporation of water, denaturation of protein, gelatinization of starch, crust formation, formation of a porous structure, browning reaction, and development of flavors. Starch gelatinization is one of the critical factors that determine the baking time and extent of gelatinization in the crumb of soft bakery products, and surface color is another important characteristic of baked products. Therefore, these could be taken as a minimum or critical baking index [40, 41]. However, partial baking is a gradual baking process. The part-baked product is a semi-finished product with an adequately rigid structure so that its preparation for consumption, handling, and storage is easy. Part-baking or partial baking is a method of bakery product manufacture consisting of two stages of baking with an intermediate storage stage (Figure 14.2).

The partial baking method is economical and time-saving as there is no need for skilled labor or baking instruments except for an oven for re-baking, and it takes a short time to obtain a fresh final product. It is very suitable for places such as restaurants, hot points in supermarkets, schools, or by domestic users, where consumers do not want to spend time and effort to perform the whole process of dough making and processing. The part-baking method, or the two-step baking procedure, can also be used successfully for bakery products such as bread, cake, and pizza (Figure 14.3). Cake and pizza are not products for daily consumption, such as bread, so long-term storage of the partially baked cake and pizza is necessary and frozen storage could be preferred over room-temperature storage [42]. In addition, the part-baking method in the bakery market could also be very advantageous, especially in special bakery products such as gluten-free bread, rich-fiber bread, and other products for different purposes. These types of products are addressed to a certain consumer group, and most of the time it is very difficult to find these products in conventional and industrial bakeries. For this

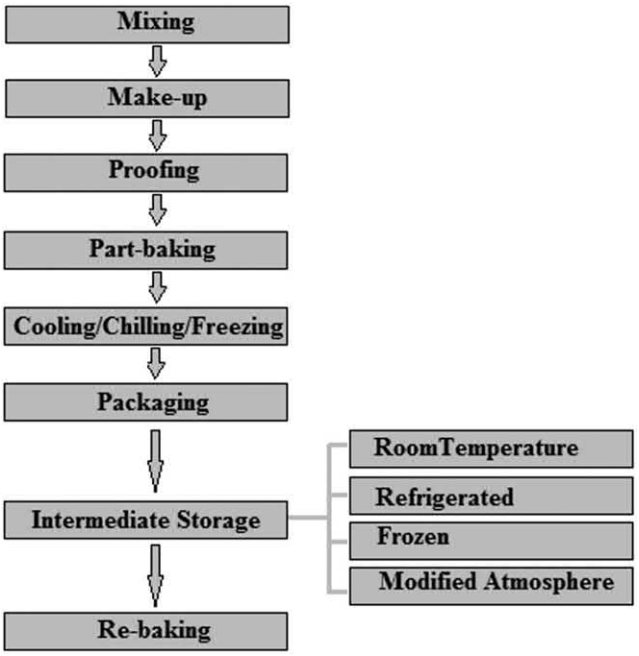

FIGURE 14.2 Flow chart of part-baked products.

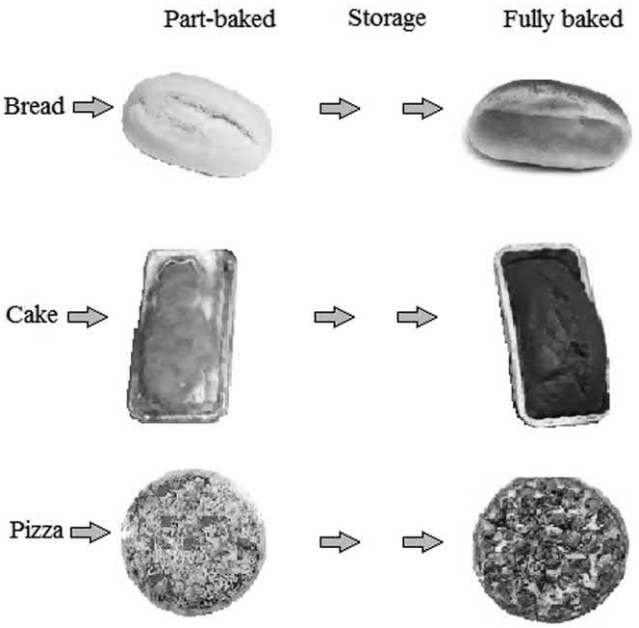

FIGURE 14.3 Part-baked and fully baked bread, cake, and pizza.

reason, the use of the part-baking process could overcome those problems and could provide a wide range of products for consumers [35, 43].

14.4.2 Part-Baked, Pre-Baking, and Post-Baking Processes

Terms such as "part-baked," "part-baking," "partially baked," and "par-bake" are used to describe a production method that involves two stages of baking (Figure 14.2). First, dough pieces with yeast are baked under the defined oven conditions into partially baked products with the minimum crust coloration, proper crumb texture, and the maximum moisture retention [33]. An increase in volume in the initial stages of prebaking is mainly caused by the expansion of the gas enclosed in the porous dough structure. The pore size in the crust may be different from the crumb, the crust having a more dense structure than the crumb [6]. In the first stage, the proper crumb texture is obtained by gelatinizing the starch and coagulating the proteins [44]. The part-baked products are cooled to a suitable temperature for packaging within the range of about 20–30°C. If the product is to be stored for a prolonged period, it is refrigerated or frozen immediately. Then, cooled and packaged part-baked products are stored up to the final re-baking at the point of sale or consumption. Finally, these part-baked products are re-baked to create an appropriate flavor and crust color similar to fresh product [23, 45]. In the household, the soft bakery products can be kept frozen, and then thawed and reheated just before consumption. Although reheating generally inverts the staling process, this is not enough to recover all the quality characteristics, like the smoothness of a freshly baked product. On the contrary, when a product is partially baked until the crumb is formed and rapidly frozen, it is possible to obtain a final product with similar characteristics to the fresh product after appropriately re-baking.

The part-baking conditions such as temperature and time have a great effect on the quality characteristics and shelf life of part-baked product [46]. Generally, a lower initial baking temperature is required to avoid excess crust coloring. However, a lower baking time efficiently extends the period of yeast activity at the beginning of the first baking. For this reason, a compensatory reduction in proof time is required. Steam may be applied during both of the baking stages [42]. Karaoglu and Kotancilar [47] stated that in relation to crumb softness, a short part-baking time was recommended for white pan bread baked in two stages (re-baking after part-baking and storage). However, Fik and Surowka [37] stated that the optimum par-baking time was between 74 and 86% of the time necessary for complete baking. The effect of baking conditions on the quality and shelf life of part-baked products is explained in detail in Section E.

14.4.3 Cooling of Part-Baked Products

Post-baking chilling or cooling of partial-baked product is an important part of the production process. Before packing, partially baked products are cooled down to storage temperature to avoid moisture condensation [48]. Physical and chemical changes occur during the cooling stage after baking [49]. When the baked products are chilled after cooking, the matrix of product that transformed from dough into crumb during baking undergoes a solidification phase due to the decrease in temperature. The heat transfer related to the cooling kinetic is highly influenced by evaporative cooling, especially at the beginning of the cooling process. This evaporative cooling corresponds to significant water loss from the product [50].

After leaving the oven, hot part-baked products must be cooled to a certain temperature to improve storage stability.

The cooling stage itself presents a spoilage risk. An appropriate cooling method should be selected at this stage because physical and chemical changes also occur during the cooling stage after baking, and these changes significantly affect the end-product quality. Therefore, a rapid technique such as vacuum cooling may provide some advantages in cooling partial-baked products. The porous structure of many bakery products is poorly conductive and causes difficulties in terms of heat removal. Vacuum cooling can be successfully used in a wide range of products, in particular with a porous water-rich matrix such as bread and cake [51]. The main advantages of this method are increased productivity, and reduced energy consumption, as well as a possible contribution to longer shelf life. Novotni et al. [48] studied the impact of barley sourdough fermentation and vacuum cooling on the shelf life and quality of composite partially baked bread during 30 days of ambient storage in modified atmosphere. They showed that vacuum cooling improved bread shape and porosity, and reduced sour taste, crust coloring, and crumbliness. Although vacuum cooling is a rapid cooling technology, it is more expensive and causes noticeable moisture loss from the products. Le Bail et al. [51] stated that vacuum chilled breads exhibited a higher moisture loss than conventionally chilled bread and that vacuum chilling had a negative effect on the texture of the bread.

14.4.4 Packaging of Part-Baked Products

The basic function of food packaging is to minimize reactions that affect the stability of packed products. In general, naturally occurring gaseous reactants (water vapor and oxygen) can seriously restrict stability under the usual food storage and distribution conditions [52]. Food packaging must protect the product from external environmental effects, such as water, water vapor, gasses, odors, microorganisms, dust, shock, vibration, compressive forces, and temperature. It should also provide compressive strength to withstand stresses and perform satisfactorily during storage and transport [53]. Each food requires different packaging characteristics due to its nature and processing steps. Therefore, packaging material must be selected by considering the balance between the barrier properties of the materials. In addition, the suitability of the form of packaging, method of preservation, and handling after purchase needs to be considered [12].

The packaging material for part-baked products must be kept in such a condition as to avoid moisture loss at a minimum. It needs to have good oxygen-barrier characteristics, physical strength against breakage at low temperature, good heat-seal ability, and low cost [54]. The most popular packaging materials applied to part-baked products are polyethylene films that are soft and flexible. Petroleum-based low-density polyethylene (LDPE) bags are a common packaging material for frozen food including frozen bread. LDPE film has characteristics of clarity, easy processing by extrusion, and low permeability to water vapor, but it is not good as a barrier for gasses, oils, or volatiles. LDPE with a density around 910 kg/m^3 has physical characteristics of softness and stretchability, and it can withstand freezing temperatures up to –70°C. However, high-density polyethylene is a good water vapor barrier, and it is an alternative approach to controlling water loss in fresh product [12, 55]. In recent years, antimicrobial packaging materials have been developed, leading to the extension of the shelf life of foods, and increased reliability is provided. In this method, antimicrobial compounds have been incorporated into packaging materials to inhibit microbial growth without compromising food quality. Antibacterial food packaging changes the atmosphere inside the package, reducing the proliferation of bacteria and thereby increasing the shelf life of the food. For this reason, antibacterial packaging materials can be used effectively for part-baked products [56–58].

14.4.5 Important Factors Affecting Quality in the Part-Baking Process

There are many factors that significantly affect the quality of part-baked products during the partial baking process, such as initial baking time and temperature, chilling, freezing, storage, and final baking (re-baking) conditions. The initial baking time of part-baked products has a significant effect on the quality of the final product. Short initial baking times at high temperatures lead to a more open structure of the crumb. The optimum prebaking time is approximately two-thirds of the time required for full baking, and at the initial baking stage, high steam is recommended for improving the crust [35]. In general, the 71% fraction of baking time for bread made it possible to obtain a product with sensory and textural qualities close to those of fresh product [37].

Chilling conditions of part-baked products, especially part-baked bread, are the most determinant parameter on the crust flaking followed by the proofing conditions. High air humidity during the chilling process generally tends to give a better crust structure and to reduce crust flaking. Also, freezing and frozen storage conditions have generally negative effects on the quality of final baked products. For this reason, different studies have been done both to optimize the processing conditions and to modify the formula to improve the quality of part-baked products [42].

The re-baking stage is compulsory to make part-baked products ready for consumption. The final baking of part-baked products is done for two main purposes; these are the coloration of the crust and the "refreshing" of the firm crumb. The second purpose corresponds to the melting of the amylopectin crystallites (at around 40–60°C) that form during storage and that result in staling. In general, partially baked products are frozen after the initial baking process and stored frozen. During the re-baking of frozen products, the heat does not penetrate to the inner parts of the crumb, and partial refreshment occurs after the second baking. For this reason, frozen part-baked products must be either partially or completely thawed by an appropriate method before the second baking. This thawing provides a uniform matrix in the second baking and a better warm-up of the inside of the crumb. In addition, the thawing process has a positive effect on crust flaking.

14.4.6 Quality Improvement Efforts in Part-Baked Products

The quality of the re-baked products after part-baking and storage is influenced by formulation and processing parameters. Bakery shortenings are a mixture of oil and solid fat at dough temperatures, and these provide specific functional properties (softness, texture, mouthfeel, structural integrity, air incorporation, heat transfer, and increase in shelf life) to bakery products [59]. Carr and Tadini [60] investigated the effect of vegetable shortening and yeast on the physical and textural properties of frozen part-baked French bread stored for 28 days. They found that higher yeast content formulations produced bread with a higher specific volume due to higher yeast activity, and the firmness and chewiness values of breads were lower in formulations containing shortening due to the softening effect, with respect to control formulations. Ferreira and Watanabe [61] showed that the use of vegetable shortening and ascorbic acid in the production of frozen part-baked French bread results in a higher specific volume.

Hydrocolloids are widely used as additives in the food industry due to their high water retention capacity. The presence of hydrocolloids retards the staling process during storage by influencing the melting, gelatinization, fragmentation, and retrogradation processes of starch, and increases water absorption. Therefore, these confer stability to products that undergo successive freeze–thaw cycles and provide multiple quality improvements in many frozen foods [62, 63]. Hydrocolloids could be also used in part-baked products that are frozen stored, and influence in a different way the characteristics of the final products according to their type. Mandala et al. [34] reported that the influence of hydrocolloid stabilizers, such as hydroxypropyl methylcellulose, xanthan, guar, and locust bean gum, on final bread characteristics was more pronounced in part-baked frozen bread than in control samples. Barcenas et al. [64] studied the influence of hydroxypropyl methylcellulose and k-carrageenan on the quality and staling of part-baked bread after frozen storage and re-baking. They concluded that the addition of hydroxypropyl methylcellulose improved the overall quality of the product during extended frozen storage and the k-carrageenan was not a good improver for the partially baked frozen bread. Hejrani et al. [65] reported that the addition of gums and enzymes to the bread recipe improves the crumb texture of the Barbari bread obtained from part-baking, frozen storage, and re-baking. In addition, the guar, amylase, and lipase interacted and increased the specific volume and the overall qualitative characteristics of the product during long frozen storage by reducing the adverse effects of that process. Conversely, they concluded that xanthan is not an appropriate improver for the part-baking process in the frozen storage of Barbari bread.

The increase in water content and in water retention of a product delays the staling of soft bakery products. Dietary fibers, such as cereal bran, have been shown to increase the water absorption of dough and the initial water content of bread and showed an enhanced effect on staling parameters, like firmness [66]. The effects of different fermentation times (90–120–420 min) and fiber contents (6% wheat bran and 6% wheat bran with 2% inulin) on the staling of bread from frozen part-baked bread were studied by Ronda et al. [66]. They reported that wheat bran had a positive effect on bread staling while inulin had the opposite effect. On the other hand, the increase in fermentation time showed a positive effect in delaying bread staling. Bignea et al. [67] have investigated the applicability of mesquite (*Prosopis alba*) flour to part-baking bread technology. They reported that breads with mesquite flour were less affected by the freezing process and frozen storage (8 weeks) in comparison with wheat breads. This would suggest the possibility of a protective effect of mesquite flour components, like fiber, against damage by freezing.

14.5 PRESERVATION OF PART-BAKED PRODUCTS

Product formulation, processing form, packaging, and storage conditions are major factors affecting the storage life of part-baked products. The desired storage life of part-baked products depends mainly on the change in their quality parameters. The chemical and physical properties of these products are the most important quality parameters as perceived by consumers [23]. Storage of partially baked product is a very important stage as its duration significantly affects the quality of the final (re-baked) product [23, 49]. Part-baked products are commercially stored at room temperature or in a freezer or refrigerator. Some undesirable changes occur during the storage of part-baked products. However, the second baking phase eliminates these undesirable changes, and the product regains its fresh textural properties [68, 69].

Partially baked products generally have very short shelf lives due to their lack of crust, light baking, and high moisture content, which increase the susceptibility to microbial growth. However, it is estimated that the storage life of part-baked products can be extended up to 6 to 12 months using various methods. Different applications, such as the use of antimicrobial preservatives, modified atmosphere packaging, infrared and ultraviolet irradiation [15], storage at refrigerator temperatures, and freezing storage, have been suggested for prolonging the shelf life of partially baked products. Packaging in a modified atmosphere containing different levels of carbon dioxide and nitrogen considerably prolongs the shelf life of a part-baked product. Storage at low temperatures (2–6°C) successfully extends the shelf life of the partially baked product up to 10 days. However, the most effective application to extend shelf life is to freeze the part-baked product and to keep the frozen product in the freezer during the storage period up to its final baking [35].

14.5.1 Room Temperature Storage

The shelf life of part-baked products stored at room temperature is limited (Table 14.2). The microbial count increases significantly during the storage of bread at room temperature if anti-microbial agents are not used [70]. After 5 and 7 days of storage, molds are visible on the crust, marking the end of the shelf life. After approximately 1 week of storage, while

TABLE 14.2
Shelf Life of Some Part-Baked Products Stored in Different Conditions

Part-Baked Product	Storage Method	Storage Temperature (°C)	Shelf Life	Reference
Sangak bread	MAP (100% CO_2)	25	21 days	Khoshakhlagh et al. [86]
Brown soda bread	40% CO_2 and 60% N_2	4	13 weeks	Leuschner et al. [69]
Gluten-free breads	Refrigerator	4	7 days	Sciarini et al. [107]
Tubers	Refrigerated storage	4	21 days	Singh et al. [111]
Bread	Freezing at –30°C	–18	12 and 20 weeks	Vulicevic et al. [23]
Bread	Refrigerator	1	28 days	Lainez et al. [72]
		7	7 days	
Flat bread	Incubator	25	3 days	Majzoobi et al. [32]
(Barbari)	Freezer	–18	60 days	
White pan bread	Incubator	20	7 days	Karaoglu et al. [70]
White pan bread	Refrigerator	4	21 days	Karaoglu et al. [70]

the crust becomes softer, the crumb becomes more crumbly and firm, and the fresh product aroma mostly disappears. Leuschner et al. [18] studied the effects of the re-baking process in part-baked soda breads on *Bacillus* spores and determined D values of *Bacillus* spores. Rope formation was observed at the end of 2 days at room temperature. The D values of spores of *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus licheniformis* isolated from breads were 14, 10, and 56 min at 100°C, respectively. However, the second baking process after storage at room temperature causes a reduction in the microbial count and promotes product freshness again [70].

Storage at room temperature is not the preferred method for the storage of partially baked products. Because the product is not fully baked, it may quickly deteriorate during storage at room temperature. Therefore, room-temperature storage can be used together with modified atmosphere packaging techniques to extend the shelf life of part-baked products. In addition, the use of antimicrobial additives causes a decrease in the numbers of total aerobic mesophilic bacteria (TAMB), coliform bacteria, *Bacillus* spores, and mold in the part-baked breads stored at room temperature [70]. Karaoglu et al. [70] studied the shelf life, microbiological, and sensory qualities of part-baked breads (i.e. white pan breads) stored at room temperature for 7 days. They reported that the numbers of TAMB, yeast, mold, and *Bacillus* spores increased before the second baking as the storage period was increased. The use of antimicrobial compounds caused a decrease in the numbers of TAMB, coliform bacteria, yeast, mold, and *Bacillus* spores in the breads. The TAMB counts of the breads without added antimicrobial were about 8-log CFU/g at the end of storage for 7 days; however, in the breads with Ca-propinate, these were 2-log CFU/g with a decrease of 6-log CFU/g. In addition, reductions of 5- and 3-log CFU/g in the numbers of *Bacillus* spores and yeast and mold counts of the breads were observed.

14.5.2 Refrigerated Storage

Refrigerated storage is a suitable alternative to preserve part-baked products since it extends the shelf life with a lower energy requirement as compared to frozen storage [46]. Therefore, partial baking and further storage at refrigerator temperatures is an effective way to delay the staling process of baked goods [71]. In fact, starch retrogradation is more rapid at refrigerator temperatures than at room temperature and is responsible for the hardening of soft bakery products. The crystallization of amylopectin can only take place when the temperature is between the glass transition temperature and the polymer melting point. Crystallization takes place in three stages, namely: nucleation, growth, and annealing of crystals. The nucleation stage is an important step because it controls crystallization. As the temperature is decreased until glass transition is achieved, the nucleation rate increases, while the growth rate of crystals increases as the temperature increases until melting temperature is reached. Recent studies show that par-baked bread stored at 1°C has a higher rate of nucleation compared with bread stored at 7°C, and a higher rate of amylopectin re-crystallization results in a faster hardening of the crumb. However, this is not a problem in the storage of partially cooked products because re-baking of the part-baked products after storage, before consumption, allows them to regain their first freshness. Therefore, the final product has the characteristics of a freshly baked product. That is, when part-baked product is re-heated to 50°C and above, the cross-linkages between starch and gluten are also easily broken and the product can return to its original freshness [9, 10].

Figure 14.4 shows the amylopectin retrogradation index produced in par-baked bread during its storage at 2°C, and in full-baked bread during its staling at 25°C. The retrogradation index of part-baked bread increases during storage at low temperatures (2°C) due to the retrogradation of amylopectin. When full (second) baking is applied, the amylopectin crystallization produced during staling was reversed due to heat, leading to a reduction of the retrogradation index. Finally, the retrogradation index increases again during the storage of full-baked bread at 25°C. This increase is not as much as in part-baked bread. Moreover, it is stated that the storage time of part-baked bread at low temperatures did not affect the

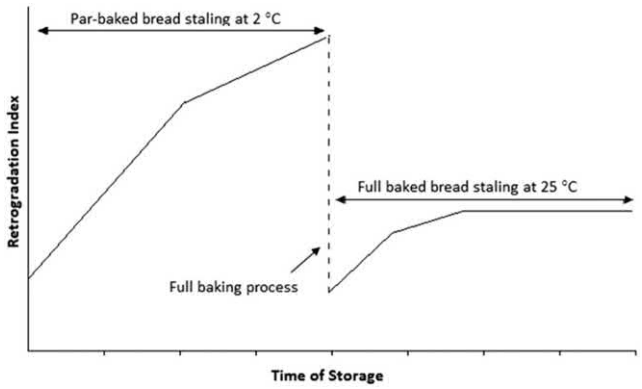

FIGURE 14.4 The retrogradation index of amylopectin during the interrupted bread-making process and storage. (From Barcenas and Roswell [9].)

crumb hardness of the full-baked counterpart, nor its further behavior during staling at 25°C [9].

The storage temperature of part-baked products significantly influences the rate of microbiological spoilage and the type of microorganism present. Refrigeration storage significantly extends the microbial shelf life of part-baked products as compared to storage at room temperature. It is stated that the microbial count of par-baked bread stored at refrigerator temperature (4°C) was lower than that of bread stored at room temperature [70]. In addition, Lainez et al. [72] noted that part-baked bread stored at 7°C presented mold growth on the 9th day, while there was no mold growth in the product stored at 1°C for 28 days.

The refrigerated storage time of part-baked bakery products generally affects baking loss, specific volume, and textural properties of the final (re-baked) product. Recent studies show that an increase in storage time of the par-baked bread under refrigeration (4°C) results in a reduction in the quality of the re-baked bread, which is related to losses in moisture content and softness and an increased baking loss [47].

Refrigerated storage leads to a lower rate of moisture diffusion from crumb to crust as compared to storage at room temperature. During the storage of a packaged product at ambient temperature, the crust becomes soft after 1 day of storage. However, with storage at refrigerator temperature, the crust keeps a certain crunchiness for more than 2 days. These crusts re-form more rapidly during re-baking than the soft crust. The re-baked crust from refrigerated product gives a better impression of freshness than the crust from a product stored at room temperature. The crust of a re-baked product stored at refrigerator temperature gives a better impression of freshness than the crust from storage at room temperature. In addition, when compared to freezing storage, it seems that storage at refrigerator temperature has some advantages. The crumb microstructure of bread stored under refrigeration is better protected as compared to that of bread stored frozen. Positive temperature storage leads to bread with better specific volume, low crumb hardness, and a slower hardening rate during staling than the bread stored as frozen [46]. However, microbial spoilage may occur in the refrigerator storage. Therefore, refrigeration in combination with modified atmosphere packaging (MAP) would generally be useful in extending the shelf life of part-baked bread [69]. In general, the use of low temperatures (2–6°C) can be successfully used for extending the shelf life of the partially baked products for up to 10 days [35].

14.5.3 Frozen Storage

Frozen storage is a process that is mostly used to preserve part-baked bakery products. To retard staling and to extend shelf life safely, the part-baked products are very often frozen and frozen stored [44, 73]. As part-baked products are baked at high temperatures, rapidly frozen at low temperatures, and then re-baked, the possibility of microbial survival is significantly reduced (Figure 14.5) [23, 74, 75]. Freezing is an excellent process for preserving food quality for prolonged shelf life because deterioration due to microorganisms and selected biochemical processes are decreased during the frozen storage. The shelf life of frozen part-baked products is much longer than that of ordinary products [43]. Gujral et al. [75] stated that the partial baking process and further frozen storage could extend the shelf life and improve the eating quality of chapati bread. However, structural changes in frozen foods occur during freezing, storage, and subsequent thawing. Although part-baked products can be frozen stored up to 12 months without microbial spoilage, the quality of the product begins to reduce after 6 weeks of frozen storage. Rapid freezing creates small ice crystals and reduces structural changes of frozen foods during storage and thawing. For this reason, controlling the freezing conditions to achieve small ice crystals is essential for frozen foods [43, 76].

The extensibility, peak force, and rupture energy of the partially baked chapati increased during prolonged frozen storage. Since soft and easily chewable chapatis with pleasing color and flavor are desirable, frozen partially baked chapati breads are considered a better option than conventionally

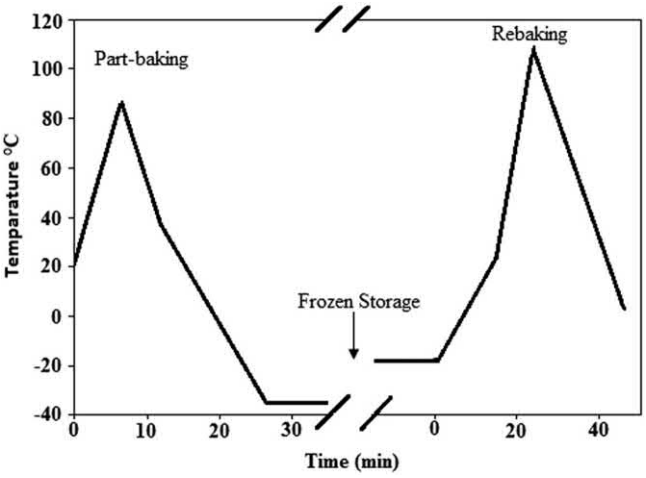

FIGURE 14.5 Temperature profile reached in the center of the crumb during a part-baking and re-baking process including frozen (intermediate) storage. (From Barcenas et al. [31].)

baked chapatis. Frozen storage is expensive due to the high maintenance costs of the cold chain as compared to storage at room and refrigerator temperatures. Under industrial conditions, the frozen part-baked bread process generally requires about 2.2 times more electrical energy than the conventional process although it may vary depending on the storage period [46, 77].

The loss of freshness in bakery products affects product quality and consumer acceptance and shortens the shelf life. Although fresh bakery products currently dominate the food market, it should be expected that in the future their importance could grow together with the consumers' increasing demands. Frozen storage is one of the most convenient and easiest of food preservation methods as compared to other commercial preservation techniques [78]. The freezing process, which can be applied before proofing or after part baking to the frozen dough or partially baked products, can increase the shelf life of product up to 12 months [35]. However, the frozen storage of part-baked products does not eliminate changes that could occur after final baking, such as interactions among the various components, which develop during the aging or storage of the re-baked product [31].

The freezing process and frozen storage temperature significantly affect the quality of fully baked and part-baked breads during storage [33]. The greater part of staling occurs in passing from 0 to 5°C with the maximum rate occurring around 4°C. A poorly performed freezing–thawing cycle can significantly increase the degree of staling undergone by the bakery product, since it passes through the optimum staling temperature twice. For this reason, during cooling of the part-baked bakery product, it must pass through this temperature range as quickly as possible, so that its quality during frozen storage is preserved. In addition, the temperature, speed, and relative humidity in the freezing process are the air parameters with greater influence on weight loss, ice concentration at the crust–crumb interface, and freezing time. The freezing time and weight loss generally decrease with the reduction in temperature and increase in air speed, whereas the ice concentration below the crust decreases with reductions in both temperature and air speed. Hence, quick freezing should be used when the objective is to minimize weight loss and freezing time, and slow freezing should be used when the objective is to minimize the ice concentration at the crust–crumb interface during the freezing process of par-baked bread [46].

The freezing treatment at temperatures above the glass transition enhances the nucleation-growth process of amylopectin crystals. Thawing and storage at higher temperatures can cause the growth and maturation of crystals and therefore an increase in the recrystallization rate as compared to unfrozen systems. The storage of fully baked and part-baked bakery products at refrigerator temperature increases the shelf life with respect to the risk of mold growth but accelerates the staling in storage at room temperature because it is in the optimum temperature range (between glass transition and melting temperature). Part-baked products at temperatures from –10 to 5°C experience maximum retrogradation of starch molecules, which in turn contributes to crumb firmness. It is, therefore, necessary to cool the part-baked product between these temperatures as quickly as possible to preserve their quality during prolonged storage [23, 33].

For all these reasons, part-baked bakery products should be stored frozen below –10°C if they need to be kept for a long time before re-baking. Immediate freezing of bakery products after baking prevents crystallization of both amylose and amylopectin and consequently slows product staling. Najafabadi et al. [33] reported that there was no significant difference among firmness of re-baked bread samples after they were part-baked and stored at –18°C compared with fresh (control) bread. However, the differences among re-baked bread samples after being part-baked and stored at 4 and 20°C are significant as compared to the fresh (control) samples. Storage at 4°C resulted in an increasing melting enthalpy of amylopectin crystallite as compared to storage at 20°C. They consequently reported that immediate freezing after baking and storage at –18°C prevented bread staling as compared to other storage temperatures.

The storage period of part-baked products under controlled freezing conditions could change the desired quality characteristics of product, such as moisture loss, starch retrogradation, loss of flavor/aroma, and increased firmness. If the part-baked products are baked adequately at the appropriate high temperature, rapidly frozen at low temperatures, and then sufficiently re-baked, the possibility of microbial survival is significantly reduced. For this reason, the desired storage life of these products generally depends on the change in their quality characteristics as mentioned above. The factors such as product formulation, processing, baking time, packaging, and storage conditions significantly affect the storage life of part-baked products [23, 79].

The freezing process and frozen storage time cause significant physicochemical changes in part-baked products, such as starch retrogradation and redistribution of the water, which have a great influence on the consumption quality of the re-baked (final) product. The freezing process itself and frozen storage can have different effects on the textural properties of re-baked (final) product compared to part-baked goods. In some studies, it was reported that the freezing process affected the quality of re-baked products more than frozen storage. However, contrasting results have also been reported by some researchers. Fik and Surowka [37] reported that the freezing process itself produces the greatest changes in sensory and textural properties of fresh bread produced from part-baked bread, while the frozen storage did not have any effect on the aging behavior. On the contrary, Barcenas et al. [31] found that the freezing process itself did not have an impact on the bread aging, whereas the time of frozen storage influenced the rate of hardening during aging, especially when prolonged frozen storage was applied.

Factors affecting the consumption quality of the part-baked products such as a reduction in specific volume, a loss of moisture content, an increase in crumb hardness, and loss of aroma can change with the increase in frozen storage time. Frozen par-baked products have generally a reduced specific volume and weight compared to fresh ones [32, 43, 80]. In

addition, long periods of frozen storage appear to be associated with a greater rate of staling [46]. Barcenas et al. [31] found a significant increase in the crumb hardness of the bread with the frozen storage time (Figure 14.6). In general, the use of baking improvers is an effective means of extending the freshness of bakery products. The baking improvers used in the full baking processes can also be directly applied to the interrupted baking process to retard staling. Barcenas et al. [81] stated that the presence of improvers such as α-amylase, k-carrageenan, and hydroxypropyl methylcellulose minimized the negative effect of the frozen storage period on staling, because these contribute to the redistribution of water and avoid interactions between starch and gluten. Therefore, baking improvers would also be useful in part-baking processes with frozen storage.

Wheat-based cereals contain both fat-soluble antioxidants, such as tocols, ferulic and caffeic acid esters and carotenoids, and water-soluble antioxidants, such as phenol acids and glycated flavonoids. Moreover, bread oxidative stability is strongly affected by temperature, presence of air or oxygen, light, and lipolytic enzymes [82]. Novotni et al. [54] stated that during frozen storage of part-baked and fully baked bread, the total phenolic content and oxidative stability decreased, but part-baked bread had longer oxidative stability than fully baked frozen bread. The researchers also noted that the oxidative stability of bread in blue-colored high-density polyethylene pouches was better than in transparent polyester-polyethylene-ethylene-vinyl alcohol copolymer pouches. This is probably due to their blue color and lower light transparency.

The freezing of bakery products is generally carried out in a single-stage process. However, a two-stage freezing process can also be used. Recently, the effects of two-stage freezing on crust flaking, total weight loss, and ice concentration in the crumb–crust interface of part-baked bread as compared to single-stage freezing were studied. It was observed that the temperature, velocity, and relative humidity of cold air are the most influential cold air parameters on the weight loss, ice concentration in the crumb–crust interface, and freezing time [83]. Moreover, it was also reported that the ice content in the interface can be considerably decreased by using slow freezing in the first stage and fast freezing in the second stage in comparison to a single-stage regime.

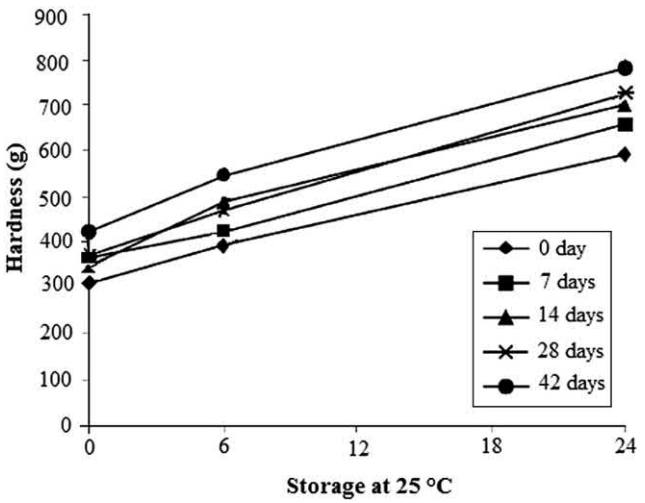

FIGURE 14.6 Effect of part-baking, freezing, frozen storage at different times, thawing, and re-baking on the crumb hardness during aging at 25°C. The different series correspond to the time of frozen storage. (From Barcenas et al. [31].)

The recrystallization of ice is observed in frozen part-baked products. It is a physical phenomenon whereby ice crystals in frozen foods increase in size but diminish numerically during frozen storage. The formation of large ice crystals through redistribution of water from small to large crystals can reduce product quality [84]. Results for microstructure observed by low-temperature scanning electron microscopy showed the physical damage suffered by the crumb constituents of par-baked bread during frozen storage. This was caused by the progressive growth of ice crystals, and this damage appears to be the main factor responsible for the loss in quality and the greater rate of staling [46].

The freezing of a non-packaged product is a faster process compared with the freezing of a packaged product. The moisture loss from non-packaged bread during freezing is negligible. Because the fast freezing process is important for the protection of product quality, it is recommended that part-baked bakery products be frozen in the non-packaged form in order to minimize the loss of quality during freezing [46]. Freezing is an expensive method of preserving, transporting, marketing, and storing foods. Moreover, cellular destruction of food could occur when foods are frozen. If freezing and frozen storage are not done correctly, the product quality can be adversely affected. The main purposes of the use of frozen partially baked products are to limit the weight loss and ice crystallization under the crust during freezing while keeping the freezing time short. To ensure that freezing is effective, it is necessary to optimize process conditions, such as air velocity, air temperature, and relative humidity [83].

14.5.4 Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is an effective method to increase the mold-free shelf life of bakery products [85]. MAP is a procedure in which the composition of air in a package of known permeability is changed after the food is filled. Several methods can be used to modify the atmosphere within the packaged product, including (i) vacuum packaging, (ii) gas packaging, and (iii) active packaging.

14.5.4.1 Vacuum Packaging

Vacuum packaging involves evacuating most of the oxygen present in the package (i.e. to a level of less than 1%). This low oxygen concentration will help to prevent the growth of aerobic organisms and to reduce the rate of oxidative rancidity. However, in the part-baking industry, vacuum packaging is not widely used because of its crushing effect on the products.

14.5.4.2 Gas Packaging

Generally, gas packaging consists of two stages. It begins with a vacuum on the product in order to remove oxygen as much

as possible from the system. Then the pack is flushed with a gas or gas mixture. Generally, gas types and concentrations in MAP systems are determined by internal factors, such as types of products, permeability of packaging material, microbial load, and product ingredients, and external factors such as temperature and storage time [86]. The common gases used are oxygen, nitrogen, and carbon dioxide. Minimum oxygen levels are used to pack food under a modified atmosphere because oxygen reacts with the foodstuff resulting in oxidative breakdown. Nitrogen is an inert gas and is used as a sterile filler gas to dilute the concentration of oxygen and carbon dioxide in the package. It acts as a cushion, thereby preventing pack collapse. Although carbon dioxide is not lethal to microorganisms, it showed both bacteriostatic and fungistatic properties and hinders the growth of certain aerobic organisms. For this reason, there is an increasing demand for the storage of bakery products in modified atmospheres, which are most often composed of carbon dioxide alone or mixtures of carbon dioxide and nitrogen [86–88].

Storage temperature fluctuations should be avoided during the storage of MAP products because changes in storage temperature had a significant effect on the characteristics of the system such as the gas composition, relative humidity, and volume of package headspace. The gas solubility, permeability of packaging, and microbial respiration can also affect headspace gas composition [86].

The possible effect of modified atmosphere packaging, ethanol content, and UV radiation dose on the mold-free shelf life of wrapped part-baked baguettes with/without chemical preservative was studied by Doulia et al. [15]. The use of modified atmosphere with 70% CO_2/30% N_2 had a very positive effect, raising the microbial shelf life of part-baked baguettes to very high levels (>70 days), as compared to the control bread which showed a shelf life of 4.6 days (i.e. 14 times) [52].

14.5.4.3 Active Packaging

Active packaging is an innovative approach to maintain or to extend the shelf life of food products while ensuring their quality, safety, and integrity. It is a good alternative to the use of chemical preservatives and MAP [89, 90]. Active packaging applications consist of two systems, namely active-scavenging (absorbers) and active-releasing (emitters). Absorbers remove undesired compounds such as moisture, carbon dioxide, oxygen, ethylene, and odor from the package environment, while emitters release desired compounds such as antimicrobial compounds, carbon dioxide, antioxidants, flavors, ethylene, and ethanol to the packaged food or into the package headspace [90].

The application of oxygen scavengers (OS) is one of the main active packaging systems in bakery products. An oxygen absorber can remove oxygen from the package headspace to a level of less than 0.01% and therefore reduce the growth of aerobic bacteria, molds, and insects, and it may prevent undesirable oxidative changes that could deteriorate the sensory quality of the bakery product [10]. For packaged part-baked bakery products, mold growth is the key factor limiting shelf life. Oxygen absorbents are three times more effective than gas packaging in improving the mold-free shelf life of bread rolls [52]. In a study, in which the palladium-based OS film in modified atmosphere (MA) packages were applied to partially baked buns, toast bread, and gluten-free bread slices, it was shown that at normal and MA conditions without OS film, visible mold growth was detected in all samples after 2 d with a simultaneous reduction in oxygen concentration in the headspace. In contrast, in MA packages with OS film, mold growth was retarded up to 8 and 10 days, resulting in a three- to four-fold longer shelf life for all types of bread tested [90].

The most commonly used absorbent to reduce the O_2 concentration in the package is Atco or Ageless. The majority of commercially available O_2 scavengers react on the basis of iron, and they actively modify the headspace and reduce the O_2 levels to less than 0.01% within 1 to 4 day(s) at room temperature. It is stated that Atco O_2 absorbers considerably extended the microbial shelf life of sliced bread, decreased O_2 concentration to below 0.1% within a few days of packaging, and did not seem to have any effect on the sensory quality of bread during storage [52].

Another example of an antimicrobial, active packaging concept is an ethanol emitter, like Ethicap. Ethicap is a sachet containing microencapsulated food-grade ethanol in a free-flowing powdery form, and it consists of 55% ethanol, 35% silicon dioxide, 10% water (all w/w), and a trace of flavors to mask the ethanol. The antimicrobial effect of ethanol as a preservative is based on ethanol's ability to lower the water activity, and it is effective in suppressing the growth of a number of bacteria, yeasts, and molds [91]. Franke et al. [91] stated that pre-baked buns, with a water activity of 0.95, packaged with different amounts of Ethicap, into gamma-sterile low-density polyethylene bags and stored at room temperatures, can be stored up to 13 days without mold growth. Typical examples of active packaging methods are presented in Table 14.3 [52].

Nanotechnologies can also be applied in order to design active packaging and are opening up new possibilities for both the food industry and consumers. Nanotechnology has been applied in the production of nanocomposites and in the nanoencapsulation of active compounds (Figure 14.7). Nanocomposites are multiphase materials characterized by a polymer (continuous phase) merged to nano-dimensional material (discontinuous phase) that can come in the form of inorganic or organic fibers, flakes, spheres, or particulates, commonly referred to as "fillers." Therefore, nanocomposites are a fusion of traditional packaging polymers with nanoparticles. Generally, the inclusion of fillers in nanoscale improves the mechanical strength of food package materials and the barrier ability against oxygen, carbon dioxide, ultraviolet radiation, moisture, and volatiles. In addition, they may allow air and other enzymes, but degrade ripening gas, such as ethylene, and have antimicrobial activity. Hence, nanocomposites may be used to extend food shelf life; thus these reduce the addition of chemical preservatives in foods. Several materials, such as metal, metal oxides, silica, clay, polysaccharide nanocrystals, chitosan, and cellulose, have been explored as fillers. Nanoencapsulation is also used to obtain antimicrobial

TABLE 14.3
Examples of Active Packaging Techniques for Bakery Products

Technique	Reagent	Packaging Form	Desirable Effect
O_2 absorber	Ferrous compounds	Sachet	Prevent mold growth
	Metallic salts	Label	Oils and fats oxidation inhibition
	Organometallic compounds	Plastic film	Elimination of insect pests
	Catechol		Flavor retention
	Pd/Pt catalysis		Retention of nutritive elements
	Glucose oxide		
	Ethanol oxidase		
Ethanol emitters	Ethanol spray	Sachet	Growth inhibition of molds and yeast
	Encapsulated ethanol		Prevent staling
	Ethanol adsorbed in silica powder		
Antimicrobial releasing	Bacteriocins (nisin, chitosan)	Sachet or film	Prevent microbial growth
	Sorbates		
	Bezoates		
	Propionates		
Antioxidants	Butylated hydroxytoluene, BHT	Film	Prevent lipid oxidation
	Butylated hydroxyanisole, BHA		
	Tocopherol		

Source: Adapted from Galic et al. [52]; Altaf et al. [112]; Day [113]; Svensson [114].

packaging systems. This technology has been applied by using essential oils that can act as potent antimicrobials, may present antifungal activity, and/or have antioxidant properties. It consists of coating essential oils within another material in the nanoscale [14].

14.5.5 Irradiation

The ionizing radiation source could be high-energy electrons, X-rays, or gamma rays, while non-ionizing radiation is electromagnetic radiation mainly from ultraviolet (UV) rays, visible light, microwaves, and infrared [92]. In particular, ionizing radiation and UV can be used to extend the microbial shelf life of part-baked products [11]. Ionizing radiation does not cause any significant rise in the temperature of the product. Therefore, the flavor, texture, and other important technological or sensory properties of most ingredients are not influenced at low doses [93]. Ionizing radiation can be used to extend the shelf life of foods by destroying yeasts and molds. The destruction of viable spoilage microorganisms can be achieved using a dose of 0.4–10 kGy, the reduction of viable non-spore forming food-borne pathogens using a dose of 0.1–8 kGy, or the sterilization of products by killing both vegetative bacteria and spores using dose levels of 10–50 kGy [20].

UV light is used in bakery products to prevent the growth of mold on the surface of fresh products. The lethal effect of UV radiation on microorganisms occurs at about 260 nm with a quantum energy of 4.9 electron volts [94]. In UV light

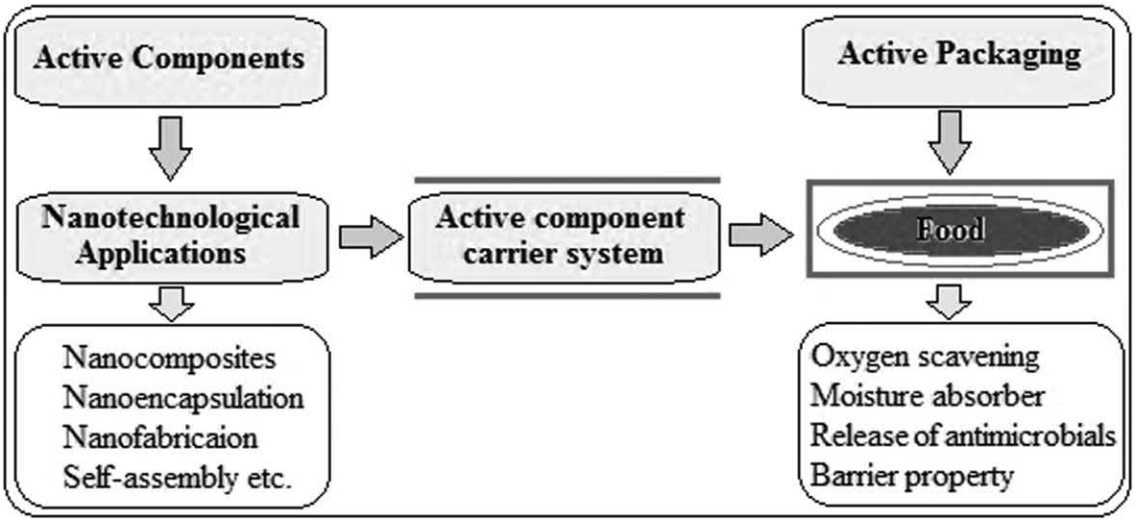

FIGURE 14.7 Active packaging and its association with nanotechnology.

processing, radiation is obtained from the UV region of the electromagnetic spectrum and classified into four wavelength ranges: UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm), and vacuum-UV (100–200 nm) [11]. UV-C has the capability of causing damage to the nucleic acid of microorganisms such as bacteria, viruses, and fungi. At a dose rate of 1000 J/m^2 or more, bacteria, yeasts, and viruses undergo as much as 4-log reductions. UV light has very low penetration depth. For this reason, it is only used for surface treatments [53]. There is direct UV irradiation of the surfaces of wrapped bakery products, which allows an extension of shelf life [11]. UV treatment can also be used to prolong the shelf life of wrapped partially baked products to minimize postbaking contamination. Doulia et al. [15] mentioned that ultraviolet treatment increased the shelf life of part-baked bread by 100%, as compared to the control, and there was no significant difference in bread shelf life among the absorbed doses of UV of 32, 81, and 162 kGy. UV light has poor penetration capacity, and therefore, a treatment of 32 kGy seems to be sufficient to decontaminate the surface of the bread, but the highest doses are not able to decontaminate the cracks and crevices.

Microwave energy is a form of electromagnetic (wave) radiation between 300 MHz and 300 GHz delivered in an enclosed cavity. Microwave heating allows the rapid and even heating of a product without major temperature gradients between the surface and the interior. Generally, a 30–60 s treatment makes wrapped bread mold-free. However, the use of this treatment is limited by the fact that it can cause condensation problems, which can adversely affect the appearance of the product [14].

14.5.6 Chemical Preservatives

Another common method to extend the microbial shelf life of part-baked products is to use chemical preservatives (antimicrobial agents) (Table 14.4). Chemical preservatives used in bakery products are calcium and sodium propionate, sorbic acid, potassium sorbate, sodium diacetate, methylparaben, propylparaben, sodium benzoate, and acetic acid at levels of 0.005 to 0.5% w/w. However, the most used chemical preservatives are propionic acid, sorbic acid, and their salts [95]. Propionic acid and calcium propionate are usually used at concentrations of 0.1 and 0.2% respectively. At these levels, they retard the growth of filamentous fungi and germination of *Bacillus* spores but do not inhibit yeast development. Sorbic acid is effective at controlling mold growth in bakery products at levels of 0.125 to 0.3% [1, 3, 96].

The storage of part-baked products at positive temperatures can cause deteriorations such as microbial and fungi growth or enzymatic reactions during storage. Therefore, it can be concluded that the use of an antimicrobial agent in part-baked bakery products is very important and, if one is not used, the part-baked breads must be stored at refrigerator temperature or in frozen storage [42]. Karaoglu et al. [70] stated that the addition of calcium propionate in the bread formulation significantly decreased TAMB, coliform bacteria, *Bacillus* spore, and yeast and mold counts, due to a decrease in the pH levels.

Adding ethanol to the complete surface of the part-baked products before wrapping, or inside the pouches before being filled with a loaf is another way to extend the shelf life of part-baked products (Table 14.5). The absorption of ethanol by the product can have a water activity-lowering effect. In addition, the great microbial inhibitory action of ethanol arises principally from the vapor phase in the headspace. Ethanol has a major effect on the structure and function of membranes, and it can disrupt cellular metabolism in diverse ways. Due to its high vapor-phase activity, ethanol has the advantage that all parts of the wrapped product are protected. It is reported that the presence of ethanol has a beneficial effect, greatly

TABLE 14.4
Common Chemical Preservatives Used in Bakery Products

Active Ingredient	Commercially Available Forms	Applications	Recommended Level (ppm)	Remarks
Propionic acid CH_3CH_2COOH	Propionic acid	Bread	2000	It is effective against molds and bacteria
	Calcium propionate	Bread (part-baked)	2000	Most effective below pH 5.5
		Soft flour tortillas	4000	Not suitable for chemical-leavened products
	Sodium propionate	Cakes, pie fillings, tortillas	2000	Compatible with baking powder Preferred in chemically leavened bakery products
Sorbic acid CH_3-CH=CH-CH=CH-COOOH	Potassium sorbate	Breads, cakes, pies	1000	Typically utilized in chemically leavened products Surface spraying for bread
Acetic acid CH_3COOH	Sodium diacetate	Yeast-leavened bakery products	3000	A synergistic anti-microbial effect with calcium propionate
Benzoic acid C_6H_5COOH	Sodium benzoate	Pie fillings	1000	Used in systems with high water activity Below pH 4.5

TABLE 14.5
Effect of Ethanol on Microbial Shelf Life of Bakery Products

Product	Treatment	Ethanol Content (%)	Storage Temperature	Mold-Free Shelf Life (Days)	Reference
White bread	Added inside the bag	0	Room temp.	4.4	Doulia et al. [15]
	Added inside the bag	1.5	Room temp.	50.7	
	Surface treatment	1.5	Room temp.	45.6	
Apple turnover	Added inside the bag	0	Room temp.	14	Smith [115]
	Added inside the bag	1.1	Room temp.	21	
Bakery product	Surface treatment	0.5	Optimal	Double increase	
	Surface treatment	1.0	Optimal	Triple increase	
Cake	Surface treatment	3	21	90	Kalathenos and Russell [116]
Muffin	Surface treatment	0.5	Room temp.	>50	Doulia et al. [117]
	Surface treatment	1.5	Room temp.	>70	

increasing the microbial shelf life of part-baked baguettes up to 50 days [15]. It is reported that ethanol also prevents or delays staling by plasticizing the protein network in the bread crumb. The anti-staling effect of ethanol has been observed in bread and flour-based confectionery products [97–99].

In a sense, the addition of an antimicrobial agent may be a waste of resources since microbial contamination of many baked products takes place primarily at the surface during the post-processing stage. Consumers mostly demand preservative-free food as well as more natural, disposable, biodegradable, and recyclable food packaging materials. Furthermore, these synthetic additives need to be declared on a product label, and this can conflict with the current consumer desire for clean labeling [79].

14.6 QUALITY DEFECTS OF PART-BAKED PRODUCTS

Some undesirable changes may occur in part-baked products stored under different conditions. These defects are crumb shrinkage, reduction/contraction in specific volume, weight loss, discoloration (dull color of the crust), separation of the crust from the crumb, and microstructure problems.

14.6.1 Crust Flaking

One of the most important quality problems in stored part-baked products is crust flaking [83], which generally occurs during the final baking stage [49]. Crust structures that are generally high in gelatinized starch content and low in evenly distributed gluten are more susceptible to cracking after the final baking, and freezing [100]. Crust flaking can be generally explained by a synergic effect of three factors: thermo-mechanical shock, drying out of the crust, and an accumulation of ice under the crust [46]. Industrial investigations show that the chilling conditions and the freezing step after partial baking are the most influential parameters in crust flaking. Generally, higher air humidity during the chilling process tends to minimize crust flaking. In addition, a higher temperature of the partially baked product on entry into the freezer seems to enhance the crust-flaking phenomenon [101].

Crust flaking in part-baked bakery products can be especially related to mechanical damage due to the intense thermomechanical shock during chilling-freezing and final baking. Depending on the hydration of the crust at the end of partial baking, the crust is affected differently by the thermomechanical strain and stresses imposed during the freezing process. Flaking does not occur after the final baking step if the part-baked products are not frozen. However, crust flakes can form even in the absence of cold storage [49, 83, 102].

The relative humidity during proving and pre-chilling is the key factor in controlling the amount of crust flaking [103]. Higher air humidity during the chilling process tends to minimize crust flaking [44]. There are two mechanisms used to explain flaking in part-baked bread. The first is the condensation of water vapor under the bread crust during the cooling phase. The mechanism by which a layer of condensed water might lead parts of the structure to become separated from the crumb has not been well explained. However, the mechanism has been based on properties or behaviors of the subsurface layer, which differ from those of the rest of the product matrix. Water crystallization between the different layers such as crust, water, and crumb might give rise to local stresses, which might be responsible for the separation of the upper crust after sudden evaporation during the final baking. The same hypothesis has been advanced to explain the formation of cracks within the crumb several millimeters or even centimeters under the crust. Considerable instantaneous evaporation of more mobile water under the upper crust during final baking might also be responsible for the detachment of the upper crust [103]. The second hypothesis is the tensile forces. The tensile forces within the bread matrix cannot relax in response to the contraction of the crumb phase during the

cooling process. It could make the bread matrix more sensitive to mechanical shocks or high hydrothermal stresses on final baking and so, in turn, would finally result in a detachment of parts of the crust and flaking phenomena [44].

Baking and storage conditions can also affect crust flaking [101]. In the production of fermented part-baked products, relative air humidity during proving and pre-chilling is the key factor in controlling the amount of flaking. In general, it is emphasized that low levels of surface hydration in these stages could favor the appearance of crust flakes [103]. However, high relative air humidity at the proving and cooling stages minimizes flaking. In other words, it can be supposed that the state of hydration of the material partly or primarily determines its capacity to deform, and the greater its level of hydration is, the more deformation it allows.

14.6.2 Shrinkage of Structure

Another problem is the crumb shrinkage developed during the chilling and freezing of part-baked products. In general, the thermo-mechanical properties of par-baked products change with freezing [46]. During freezing, the frozen superficial crust is exposed to a negative tangential stress that could explain the crust-flaking phenomena because of this crumb shrinkage. Actually, these tensions and stresses act in the crust–crumb interface in which extreme gradients in terms of temperature, moisture, and frozen water occur [44]. Crystallization of the amylose during cooling and partial recrystallization of the amylopectin during freezing and frozen storage could clarify the crumb shrinkage [46]. Reduction of the crumb shrinkage is an important issue as it causes crust flaking. Specific enzymes and additives combined with appropriate processing conditions such as a specific cooling and freezing rate should be permitted to reach this goal [44]. Almeida and Chang [104] reported that the structural problem of pre-baked bread could be overcome through joint adoption of the following parameters: (i) carrying out a more open modeling, (ii) ending the proofing stage when the dough is resistant to touch, (iii) establishing a good par-baking stage (only a few seconds of water steam at the beginning of baking), (iv) appropriate baking time and temperature, (v) last moments realized at dry conditions (steam elimination), and (vi) ending only when the center of the bread reaches 93–96°C.

14.6.3 Reduction in Specific Volume

Although some studies revealed that the storage period negatively affects the part-baked product volume [32, 80, 105, 106, 107], some others showed no significant relationship between storage period and volume [64, 108]. Karaoglu et al. [80] showed that the increase in the frozen storage time of par-baked cake leads to a decrease in the quality of the re-baked cake, namely an increase of baking loss and cake crumb firmness, and a loss in the moisture content and specific volume. Majzoobi et al. [32] studied the influence of storage (at ambient, 25°C and freezing, –18°C temperatures) on the quality and staling of part-baked flat bread (Barbari). They reported that the volume of full-baked breads, after part-baking and storage, decreased over the storage time at both ambient and freezing temperature (Figure 14.8). However, Almeida and Chang [108] stated that specific volume, springiness, and sensory evaluation of re-baked rolls practically did not change with the frozen storage period.

14.6.4 Weight Loss

If the bakery products are frozen without packaging, weight losses occur due to water evaporation during pre-chilling. Water loss of frozen part-baked products is higher than in the case of a conventional process. The evaporation amount is related to the difference in partial vapor pressure between the surface of the product and the ambient air. The lower the freezing rate is, the slower reduction is in surface temperature, and this causes the difference in vapor pressure between food and

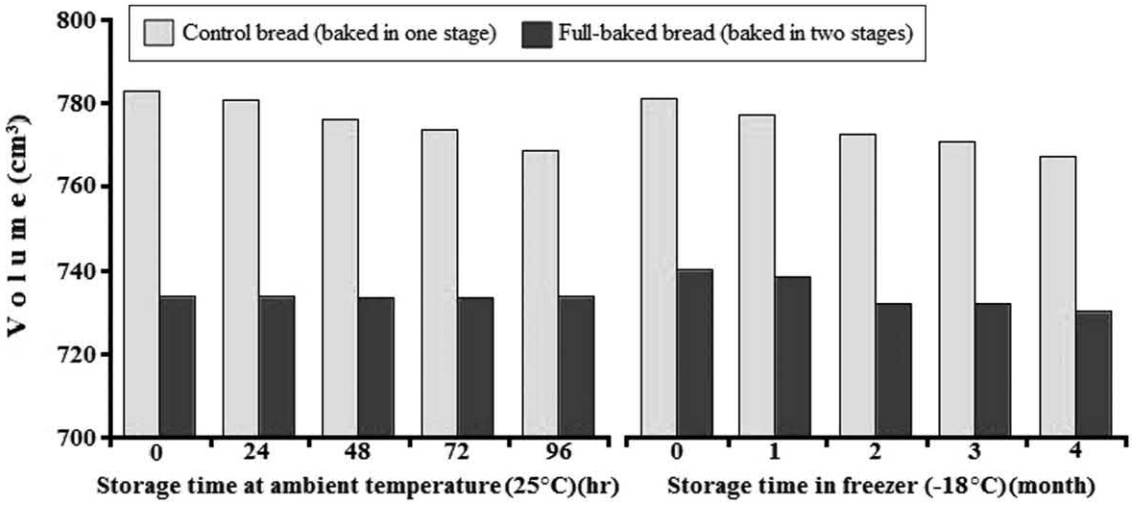

FIGURE 14.8 Volume of control and full-baked bread samples stored at ambient temperature and in freezer. (Revised from Majzoobi et al. [32].)

air to remain large for a longer time. At the same time, a low freezing rate would cause the prolongation of freezing process duration. Eventually, both factors increase the weight loss of frozen products. The weight loss by dehydration in products frozen in this way is approximately 2–3% [83]. Figure 14.9 shows the baking weight loss of the re-baked breads obtained from part-baked breads and part-baked brown soda bread during freezing storage and re-baking at in-oven temperatures of 180 and 200°C. The nonfrozen samples have lower baking weight loss compared with the frozen samples. In addition, a linear relationship between baking loss and length of the second baking phase was observed. The moisture loss for the higher oven temperature increases as compared to the lower re-baking temperature [43, 69].

Weight loss can be overcome by reducing the post-baking–chilling time and by starting the freezing earlier or by increasing the air humidity during chilling. In addition, a higher temperature of the partially baked product upon entrance to the freezer can enhance the crust-flaking phenomenon. Therefore, the temperature of the pre-baked product at the start of freezing should not be too high. The final baking is generally combined with thawing. The lower baking temperature results in higher water loss and longer baking time. For this reason, a fast final baking time at a high temperature is usually used for these products to decrease weight loss [101].

14.6.5 Discoloration

Another problem caused by frozen storage is the appearance of snow-white discoloration of the crumb. The appearance of discoloration generally occurs after a relatively long time in the freezer. After thawing the product, the white rings become weaker but do not disappear. The formation of this white ring or freezing ring is due to the drying of the crumb just under the crust during frozen storage. Researchers have reported that this discoloration (white rings) of the crust is caused by the transference of moisture by sublimation and diffusion from the highly moist center of the crumb to the low moisture-content region of the crust [46].

14.6.6 Separation of the Crust from the Crumb

During baking of bakery products, there is a small empty space that separates the crust from the crumb. This separation has the following effects: (i) during baking, the heat transfer from the crust to the crumb is slower, (ii) the crust can be baked more thoroughly than the crumb, and (iii) after baking, diffusion of the moisture from the crumb to the crust is slower. Freezing of bakery products can make more obvious the problem of the separation of the crust from the rest of the product. This is especially true for crisp bread like baguettes in which the most obvious manifestation of the quality problem is the separation of the crust from the crumb. Because, in crispy bread, there is a considerable difference in moisture content between the crust and the crumb, expansion and contraction occur at different rates during the freezing and thawing processes. This creates remarkable pressure on the structure of the product, and the crust is separated from the crumb due to various stresses and deformations. This problem could be solved by the migration of moisture from the crumb to the crust, which would subsequently leave it softer and more flexible, but this could be unacceptable for a crisp product [46].

14.6.7 Microstructure Problems

The internal microstructure of bakery products plays an important role in maintaining quality, since the physicochemical,

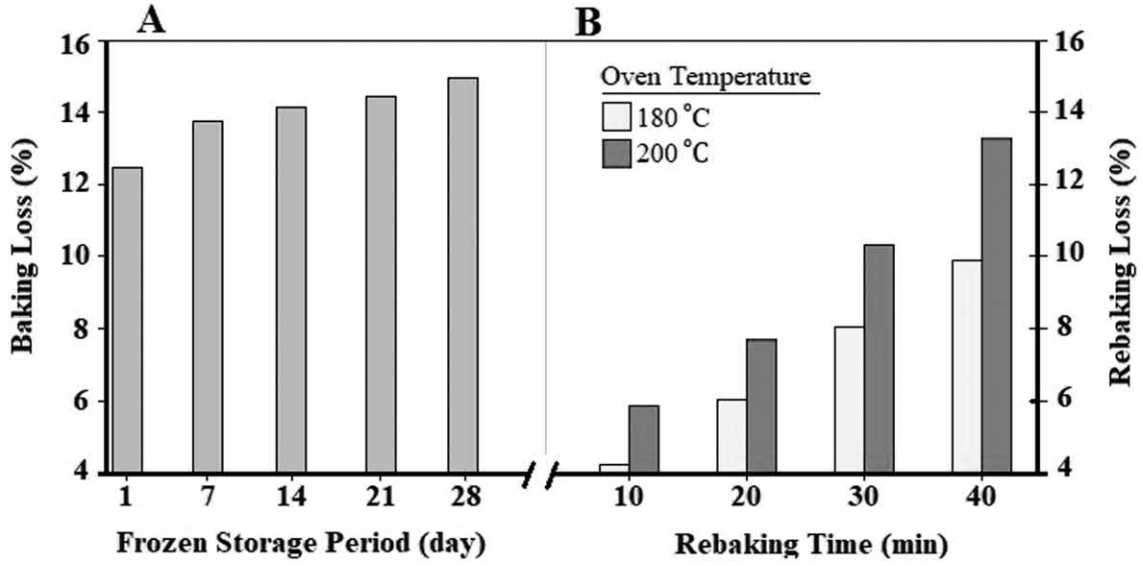

FIGURE 14.9 Baking loss of the re-baked breads obtained from part-baked breads during frozen storage (A) and baking loss of re-baked breads obtained from part-baked brown soda bread during re-baking at different oven temperature (B). (Adapted from Majzoobi et al. [43]; Leuscher et al. [69].)

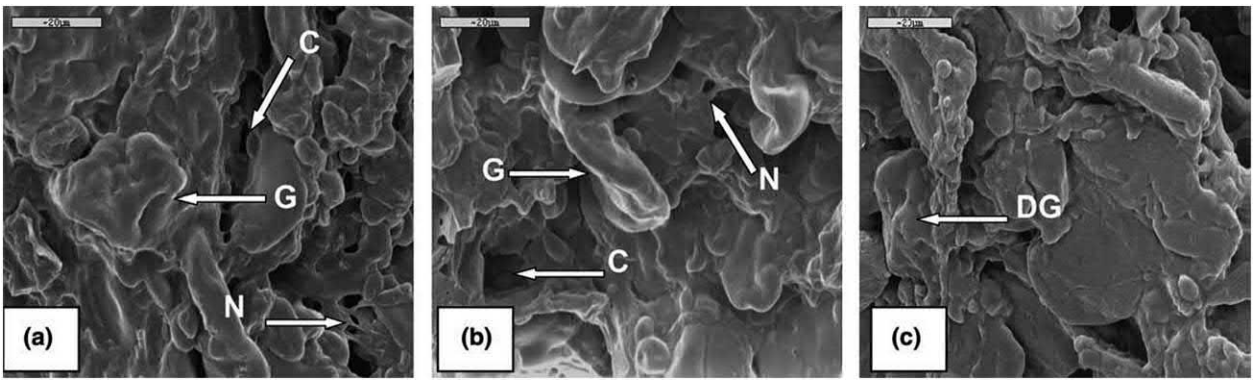

FIGURE 14.10 Cryo-SEM micrographs of gas cell walls (1500X) of bread from conventional breadmaking process (a), bread crumb from par-baked bread after freezing, thawing, and baking (b), and crumb bread from par-baked frozen bread after 42 days of frozen storage (c). The arrows show starch granules (G), cavities (C), protein network (N), and damaged granules (DG). (From Barcenas and Rossell [74].)

sensory, and transport properties of these foods are largely dependent on crumb structure, and crumb construction affects textural properties of bakery products [109]. Freezing results in the disrupted microstructure of crumb, mainly the protein matrix, bringing about the rupture of the denatured gluten network and the subsequent release of the bound water, and some damage and deformed starch granules. The faster the freezing rate is, the less damage the microstructure of the food undergoes. The effect of frozen storage time on the microstructure of the par-baked bread crumb has been studied by Barcenas and Rosell [74]. Cryo-SEM micrographs of gas cell walls of bread crumb are shown in Figure 14.10. The microstructure results from cryo-SEM might indicate that the physical damage undergone by the crumb constituents of the part-baked bread during frozen storage is caused by the progressive growth of the ice crystals. This damage seems to be the main cause of quality loss and the greater rate of aging.

14.7 CONCLUSION

Bakery products are the most widely consumed foods in the world and consumers generally desire to eat them fresh. However, most of them have a short shelf life, resulting from undesired changes that begin just after the baking process. Soft bakery products are dynamic systems that undergo physical, chemical, and microbiological changes during processing and storage, which limit their shelf life. Storage temperature, relative humidity, packaging material and the gaseous environment surrounding the product, level of preservatives, pH, and especially the moisture content and a_w significantly affect the spoilage problems in baked products. Basically, there are three important methods to extend the shelf life of bakery products. These are frozen dough technology, freezing of bakery products, and the production of partially baked products. The partial baking process allows fresh products to be available at any time of the day. This method is economical and time-saving as there is no need for skilled labor or baking instruments except for an oven for re-baking, and it takes a short amount of time to obtain a fresh final product at any time. Part-baking or partial baking is a method of producing baked products involving two stages of baking with an intermediate storage stage. Storage conditions are the major factors affecting the storage life of part-baked products. The desired storage life of part-baked products depends mainly on the change in their quality parameters such as chemical, physical, and microbiological. Partially baked bakery products generally have a very short shelf life due to their lack of crust, light baking, and high moisture content, which increase the susceptibility to microbial growth. However, it is estimated that the storage life of part-baked products can be extended up to 6 to 12 months by using various methods. Different applications such as the use of antimicrobial preservatives, modified atmosphere packaging, infrared and ultraviolet irradiation, storage at refrigerator temperature, and freezing storage have been suggested for prolonging the shelf life of partially baked products.

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15 Hurdle Technology (Combined Methods) for Food Preservation: Theory and Basic Aspects

Lothar Leistner and Mohammad Shafiur Rahman

CONTENTS

15.1 INTRODUCTION

The microbial stability and safety of most traditional and novel foods depend on a combination of several preservative factors (called hurdles), which the microorganisms present in the food are unable to overcome. This is the so-called hurdle effect, first introduced by Leistner [1]. Figures 15.1 and 15.2 show the visualized concept of hurdle technology, and these indicate when the microbe passed over the hurdles or stairs, and when it was exhausted and unable to perform its physiological process or died. The hurdle effect is of fundamental importance for the preservation of foods, since the hurdles in a stable product control microbial spoilage and food poisoning as well as desired fermentation processes [1, 2]. Leistner and co-workers acknowledged that the hurdle concept illustrates only the well-known fact that complex interactions of temperature, water activity, pH, redox potential, etc., are significant for the microbial stability of foods. From an understanding of the hurdle effect, the hurdle technology [3] is derived, and it allows improvements in the safety and quality of foods, by deliberate and intelligent combinations of hurdles. Over the years, the insight into the hurdle effect has been broadened and the application of hurdle technology extended. In industrialized countries, hurdle technology is currently of particular interest for minimally processed foods, whereas in developing countries it is of interest for foods storable without refrigeration, due to stabilization by hurdle technology; thus at present hurdle technology has paramount importance. The application of deliberate and intelligent hurdle technology is now advancing rapidly worldwide. This concept is synonymously called food preservation by combined methods, combined processes, combination preservation, or combination techniques. Special expressions for this concept have been coined in different languages, for example Hürden-Technologie in German, hurdle technology in English, technology des barrières in French, tecnologia degli ostacoli in Italian, tecnologia de obstaculos in Spanish, zanglangishu in Chinese. Recently, the term hurdle technology has been most often used.

In Europe, a three-year research project on "Food Preservation by Combined Processes," supported by the European Commission, to which scientists from 11 European countries have contributed, fostered the application of hurdle technology [4, 5]. The hurdle technology concept proved successful, since an intelligent combination of hurdles secures microbial stability and safety as well as the sensory quality of foods [6–8]. It provides convenience and freshness of foods to consumers and is cost-efficient for the producers since it demands less energy during production and storage.

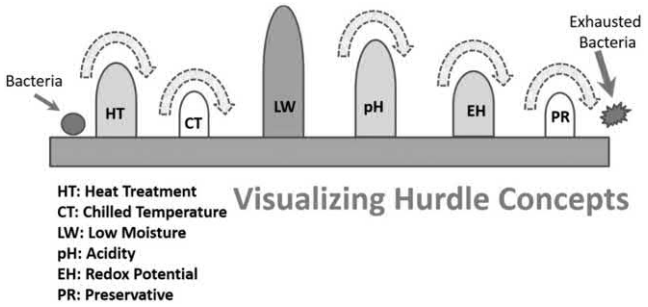

FIGURE 15.1 Visualization of hurdles on microorganism considering each preservation effect is a hurdle.

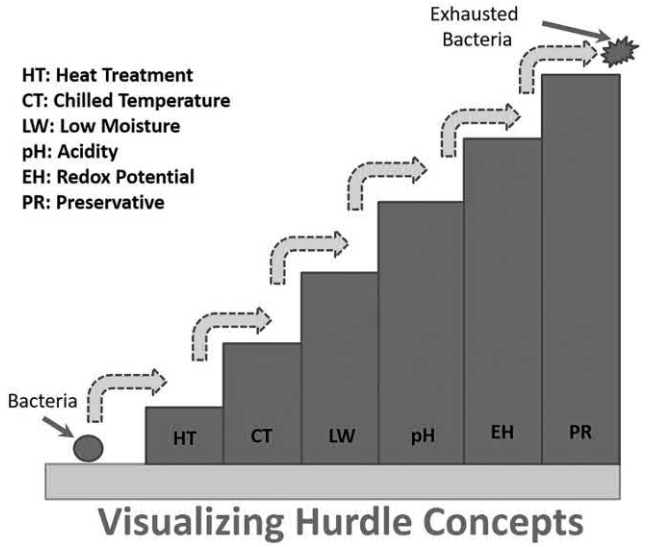

FIGURE 15.2 Visualization of hurdles on microorganism considering each preservation effect is a stair.

15.2 PRINCIPLES OF COMBINED PRESERVATION METHODS

There are many preservation methods used for making foods stable and safe, e.g. heating, chilling, freezing, freeze-drying, drying, curing, salting, sugar-addition, acidification, fermentation, smoking, and oxygen removal. However, these processes are based on relatively few parameters or hurdles, i.e. high temperature (F value), low temperature (t value), water activity (a_w), acidification (pH), redox potential (E_h), preservatives, and competitive flora. In some of the preservation methods mentioned, these parameters are of major importance; in others they are only secondary hurdles [1, 6, 9, 10].

The critical values of these parameters for the death, survival, or growth of microorganisms occurring in foods have been determined in recent decades, and are now the basis of food preservation. However, the critical value of a particular parameter changes if other preservative factors are present in the food. For instance, the heat resistance of bacteria increases at low a_w and decreases in the presence of some preservatives, or a low E_h increases the inhibition of microorganisms caused by a reduced a_w. The simultaneous effect of different preservative factors could be additive or even synergistic. Furthermore, as mentioned before, the microbial stability and safety of many foods are based on the combined effects of hurdles. For instance, mildly heated canned foods ("half-preserved" or "three-quarter-preserved") need refrigeration during storage, or fermented sausages are only stable and safe if both the a_w and the pH are in an appropriate range. Therefore, in food preservation the combined effect of preservative factors must be taken into account, which is illustrated by the so-called hurdle effect.

15.2.1 Hurdle Effects

For each stable and safe food, a certain set of hurdles is inherent, which differ in quality and intensity depending on the particular product; however, the hurdles must keep the "normal" population of microorganisms in this food under control. The microorganisms present ("at the start") in a food should not be able to overcome ("leap over") the hurdles present, otherwise the food spoils or even causes food poisoning. In previous publications [6, 7], some examples were given to illustrate the hurdle effect, which are quite helpful for an understanding of the hurdle effect, as well as for the applications of combined methods in food preservation.

Figure 15.3 gives eight examples of the hurdle effect. Example 1 represents a food that contains six hurdles: high

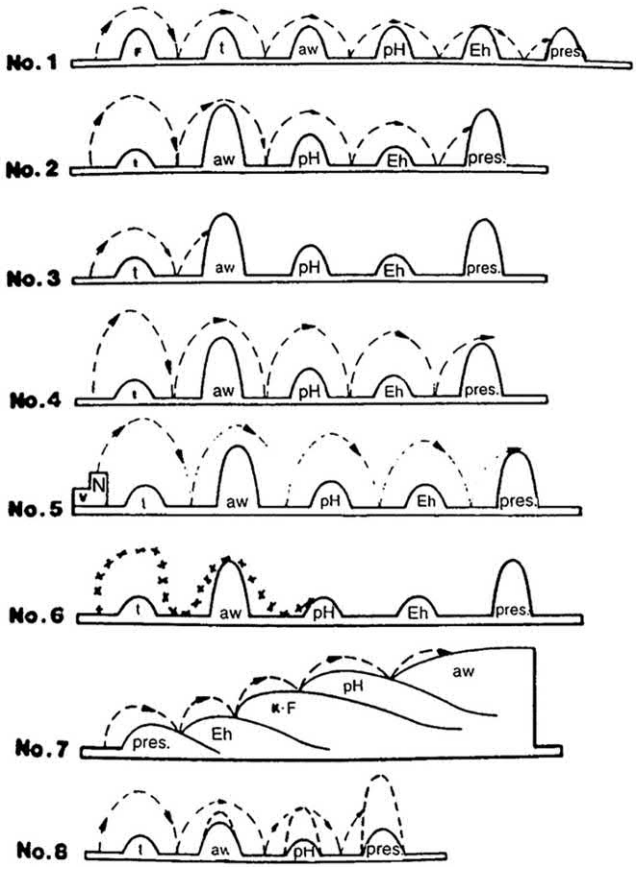

FIGURE 15.3 Illustration of the hurdle effect, using eight examples. Symbols have the following meanings: F, heating; t, chilling; a_w, water activity; pH, acidification; E_h, redox potential; pres., preservatives; K-F, competitive flora; V, vitamins; N, nutrients. (From Leistner [6, 8].)

temperature during processing (F value), low temperature during storage (t value), water activity (a_w), acidity (pH), redox potential (E_h), and preservatives (pres.). The microorganisms present cannot overcome these hurdles, and thus the food is microbiologically stable and safe. However, example 1 is only a theoretical case, because all hurdles are of the same height, i.e. have the same intensity, and this rarely occurs. A more likely situation is presented in example 2, since the microbial stability of this product is based on hurdles of different intensity. In this particular product the main hurdles are a_w and preservatives, whereas other less important hurdles are storage temperature, pH, and redox potential. These five hurdles are sufficient to inhibit the usual types and numbers of microorganisms associated with such a product. If there are only a few microorganisms present at the start (example 3), then a few or only low hurdles are sufficient for the microbial stability of the product. The ultra-clean or aseptic processing of perishable foods is based on this principle. The same proves true if the initial microbial load of a food (e.g. high-moisture fruits or carcass meat) is substantially reduced (e.g. by application of steam), because after such a reduction fewer microorganisms are present at the start, which are easier to inhibit using few or low hurdles. On the other hand, as in example 4, if due to bad hygiene conditions too many undesirable microorganisms are initially present, even the usual inherent hurdles in a product may be unable to prevent spoilage or food poisoning. Example 5 is a food rich in nutrients and vitamins, which foster the growth of microorganisms (this is called the "booster" or "trampoline effect"), and thus the hurdles in such a product must be enhanced, otherwise they will be overcome. Example 6 illustrates the behavior of sublethally damaged microorganisms in food. If, for instance, bacterial spores in meat products are damaged sub-lethally by heat, then the vegetative cells derived from such spores lack "vitality," and therefore are inhibited by fewer or lower hurdles. In some foods, stability is achieved during processing by a sequence of hurdles, which are important in different stages of the ripening process and lead to a stable final product. Example 7 illustrates a sequence of hurdles in fermented sausages. Finally, example 8 illustrates the possible synergistic effect of hurdles, which probably relates to a multi-target disturbance of the homeostasis of microorganisms in foods.

15.2.2 Hurdle Technology

The better understanding of the occurrence and interaction of different preservative factors (hurdles) in foods is the basis for improvements in food preservation. If the hurdles in a food are known, the microbial stability and safety of this food might be optimized by changing the intensity or quality of these hurdles. Therefore, from an understanding of the hurdle effect, hurdle technology has been derived [3], which means that hurdles are deliberately combined in the preservation of traditional and novel foods. By an intelligent mix of hurdles, it is possible to improve not only the microbial stability and safety but also the sensory and nutritive quality as well as the economic properties of a food. For example, it is important how much water in the product is compatible with its microbial stability, and if an increased a_w is compensated by other hurdles (pH, E_h, etc.) this food becomes more economical. Even the pet food industry now employs this principle. A stable pet food was formerly produced with a_w of 0.85, and this needed the addition of excessive amounts of propylene glycol, which might have caused health implications for cats. With the application of hurdle technology, pet foods are microbiologically stable at ambient temperatures with a_w of 0.94, and these are healthy, tasty, and economic [6]. Hurdle technology is increasingly used for food design in industrialized and developing countries for optimizing traditional foods and for making new products according to needs. For instance, if energy preservation is the goal, then energy-consuming hurdles such as refrigeration are replaced by other hurdles (a_w, pH, or E_h) which do not demand energy and still ensure a stable and safe food [1]. Furthermore, if we want to reduce or replace preservatives, such as nitrite in meats, we could emphasize other hurdles in the food, e.g. a_w, pH, refrigeration, or competitive flora, which would stabilize the product [11].

15.2.3 Total Quality

Stanley [12] proposed that the hurdle technology approach might be applicable to a wider concept of food preservation than just microbial stability, but in order for it to work, a precise knowledge of the effectiveness of each hurdle for a given commodity is required. Furthermore, he suggested distinguishing between positive and negative hurdles for the quality of foods. Certainly, hurdle technology is applicable not only to safety, but also to quality aspects of foods, although this area of knowledge has been much less explored than the safety aspect. McKenna [13] emphasized that while hurdle technology is appropriate for securing the microbial stability and safety of foods, the total quality of foods is a much broader field and encompasses a wide range of physical, biological, and chemical attributes. The concept of combined processes should work towards the total quality of foods rather than the narrow but important aspects of microbial stability and safety. At present, the tools for applying hurdle technology to total food quality are still not adequate, and this is equally true for predicting food quality by modeling. However, researchers should appreciate the wider power of the hurdle technology concept, and the food industry should use the available tools of combined processes for as many quality enhancements as possible [13].

Some hurdles, e.g. Maillard reaction products, influence the safety as well as the quality of foods, because they have antimicrobial properties and at the same time improve the flavor of the products, and this also applies to nitrite used in the curing of meat. The possible hurdles in foods might influence the stability and safety, as well as the sensory, nutritive, technological, and economic properties of a product, and the hurdles present might be negative or positive for securing the desired total quality of a food. Moreover, the same hurdle could have a positive or a negative effect on foods, depending on its intensity. For instance, chilling to an unsuitable

low temperature will be detrimental to fruit quality ("chilling injury"), whereas moderate chilling is beneficial. Another example is the pH of fermented sausages, which should be low enough to inhibit pathogenic bacteria, but not so low as to impair taste. If the intensity of a particular hurdle in a food is too small, it should be strengthened; on the other hand, if it is detrimental to the total food quality it should be lowered. By this adjustment, the hurdles in foods should be kept in the optimal range, considering safety as well as quality [7, 14].

15.2.4 Potential Hurdles

For the advanced application of hurdle technology, a continually increasing number of preservative factors (hurdles) become available (Table 15.1). The most important hurdles in common use for the preservation of foods, either applied as "process" or "additive" hurdles, are temperature (high or low), decreased water activity (a_w), acidity (pH), low redox potential (E_h), preservatives (e.g. nitrite, sorbate, sulfite), and competitive microorganisms (e.g. lactic acid bacteria). However, in addition, more than 50 hurdles of potential use for foods of animal or plant origin, which improve the stability and/or the quality of these products, have hitherto been identified and described [14, 15], and the list of possible hurdles for food preservation is by no means complete. At present especially physical, non-thermal processes (e.g. high hydrostatic pressure, mano-thermo-sonication, oscillating magnetic fields, pulsed electric fields, light pulses) receive considerable attention, since in combination with conventional hurdles they are of potential use for the microbial stabilization of fresh-like food products with little induced degradation of sensory and nutritional properties. With these novel processes, often only a reduction of the microbial load is intended (i.e. not complete sterility), and the growth of the remaining microorganisms is inhibited by additional, conventional hurdles. Another group of hurdles which is at present of special interest in industrialized as well as in developing countries are "natural preservatives" (e.g. spice extracts, lysozyme, chitosan, pectin hydrolysate, protamine, paprika glycoprotein, hop extracts). Moreover, the microstructure of some foods (e.g. emulsions, fermented sausages, ripened cheese) might be a considerable hurdle in relation to microbial growth. However, not all of the potential hurdles for food preservation are commonly applied, and certainly not all of them to the same food product.

TABLE 15.1
Incomplete List of Potential Hurdles for Foods of Animal or Plant Origin, Which Improve the Stability and/or the Quality of These Products

Hurdle	Possible Options
Temperature	Low or high
pH	Low or high
a_w	Low or high
E_h	Low or high
Modified atmosphere	Nitrogen, carbon dioxide, oxygen
Packaging	Aseptic packaging, vacuum or modified atmosphere or active packaging, edible coatings
Pressure	High
Radiation	Microwave, ultraviolet light (UV), irradiation
Other energy	Nano-thermo-sonication, high electric field pulses, oscillating magnetic field pulses, radiofrequency energy, photodynamic
Microstructure	Emulsions, fermented sausage, ripened cheese
Competitive flora	Lactic acid bacteria
Preservatives	Organic acids, lactate, acetate, sorbate, ascorbate, glucono-delta-lactone, phosphates, propylene glycol, diphenyl, parabens, free fatty acids and their esters, phenols, monolaurin, chelators, Maillard reaction products, ethanol, spices and their extracts, nitrite, nitrate, sulfite, carbon dioxide, oxygen, ozone, chlorine, smoke, antioxidants, pimaricin and other antibiotics, lysozyme, chitosan, lactoperoxidase, nisin and other bacteriocins, pectine hydrolysate, protamin, hop extracts

Source: Leistner [14], Bogh-Sorensen [15].

15.3 BASIC ASPECTS

Food preservation implies putting microorganisms in a hostile environment, in order to inhibit their growth, shorten their survival, or cause their death. The feasible responses of the microorganisms to such a hostile environment determine whether they may grow or die. Related to these responses more basic research is needed, because a better understanding of the physiological basis for the growth, survival, and death of microorganisms in food products might open new dimensions for food preservation [7]. Furthermore, such an understanding would be the scientific basis for an efficient application of hurdle technology in the preservation of foods. Recent advances in these respects have been made by considering the homeostasis, metabolic exhaustion, and stress reactions of microorganisms, as well as by introducing the concept of multi-target preservation for a gentle but effective preservation of foods [3, 11].

15.3.1 Homeostasis

A key phenomenon, which deserves more attention in food preservation, is the interference by the food with the homeostasis of microorganisms [16]. Homeostasis is the tendency to uniformity or stability in the normal status (internal environment) of organisms. For instance, the maintenance of a defined pH within narrow limits is a feature and prerequisite of living organisms [17], and this applies to higher organisms as well as to microorganisms. The homeostasis in higher organisms at the molecular, subcellular, cellular, and systematic levels are known in the fields of molecular biology, biochemistry, physiology, pharmacology, and medicine [17]. This knowledge should be transferred to microorganisms important in the poisoning and spoilage of foods. If the homeostasis of microorganisms, i.e. their internal

equilibrium, is disturbed by preservative factors (hurdles) in foods, they will not multiply, i.e. they will remain in the lag-phase or even die, before their homeostasis is re-established ("repaired"). Thus, food preservation is achieved by disturbing the homeostasis of microorganisms in a food temporarily or permanently [7].

Gould [16] has pointed out that during evolution a wide range of more or less rapidly acting mechanisms (e.g. osmoregulation to counter-balance a hostile water activity in food) have developed in microorganisms that act to keep important physiological systems operating, in balance, and unperturbed even when the environment around them is greatly perturbed [18]. In most foods the microorganisms operate homeostatically, in order to react to environmental stresses imposed by the preservation procedures applied, and the most useful procedures employed to preserve foods are effective in overcoming the various homeostatic mechanisms that the microorganisms have evolved in order to survive extreme environmental stresses [18]. The repair of a disturbed homeostasis demands much energy, and thus the restriction of energy supply inhibits the repair mechanisms of the microbial cells and leads to a synergistic effect of preservative factors (hurdles). Energy restrictions for microorganisms are caused by anaerobic conditions, such as in "vacuum" or in "modified atmosphere" packaging of foods. Therefore, a low a_w (and/or a low pH) and low redox potential of foods act synergistically [18]. The interference with the homeostasis of microorganisms or entire microbial populations forms an attractive and logical focus for improvements in food preservation techniques [18].

15.3.2 Metabolic Exhaustion

Another phenomenon of certainly practical importance is the metabolic exhaustion of microorganisms, which could lead to an "autosterilization" of foods (i.e. decrease of bacteria with storage time instead of their growth) (Figure 15.4). This was first observed by us and initially not believed, many years ago [19], with mildly heated (95°C core temperature) liver sausage adjusted to different water activities by the addition of salt and fat; the product was inoculated with *Clostridium sporogenes* PA 3679 and stored at 37°C. Clostridial spores, which survived during the heat treatment, vanished in the product during storage, if the products were stable. Later this behavior of *Clostridium* and *Bacillus* spores was regularly observed during the storage of shelf-stable meat products (SSP), especially F-SSP [20]. The most likely explanation is that bacterial spores, which survive the heat treatment, are able to germinate in these foods under less favorable conditions than those under which vegetative bacteria are able to multiply [6]. Therefore, during storage of these products some viable spores germinate, but the germinated spores or vegetative cells derived from these spores die. Thus, the spore counts in stable hurdle technology foods actually decrease during storage, especially in unrefrigerated foods. During studies in our laboratory with Chinese dried meat products, we observed the same behavior of microorganisms [21]. If these meats were contaminated after processing with staphylococci, salmonellae, or yeasts, the counts of these microorganisms in stable products decreased quite quickly during unrefrigerated storage, especially in meats with a water activity close to the threshold for microbial growth. The same phenomenon was observed by Latin American researchers [22–25] in their studies with high-moisture fruit products (HMFP). A variety of bacteria, yeasts, and molds, which survived the mild heat treatment, decreased quite quickly in the products during unrefrigerated storage, because the hurdles applied (pH, a_w, sorbate, sulfite) did not allow growth.

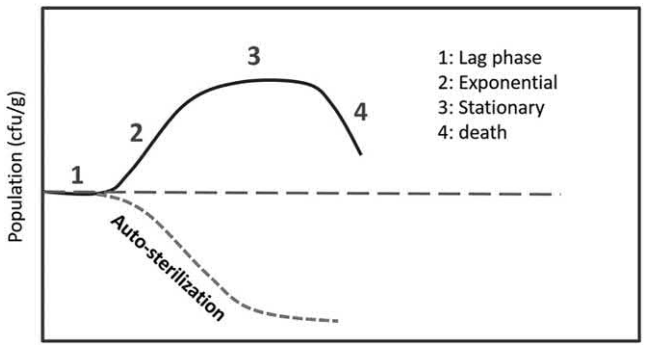

FIGURE 15.4 Microbial population decreasing during storage as a result of auto-sterilization.

A general explanation for this behavior might be that vegetative microorganisms which cannot grow will die, and they die more quickly if the stability is close to the threshold for growth, the storage temperature is elevated, antimicrobial substances are present, and the organisms are sub-lethally injured (e.g. by heat) [7]. Apparently, microorganisms in stable hurdle technology foods strain every possible repair mechanism for their homeostasis in order to overcome the hostile environment; by doing this they completely use up their energy and die, if they become metabolically exhausted. This leads eventually to an autosterilization of such foods [26]. Thus, due to autosterilization, hurdle technology foods, which are microbiologically stable, become even safer during storage, especially at ambient temperatures. For example, salmonellae, which survive the ripening process in fermented sausages, vanish more quickly if the products are stored at ambient temperature, and they will survive longer and might then cause food poisoning if the products are stored under refrigeration [7]. Salmonellae survive in mayonnaise at chill temperatures much better than at ambient temperature. Unilever laboratories in Vlaardingen have confirmed metabolic exhaustion in water-in-oil-emulsions (resembling margarine), which were inoculated with *Listeria innocua*. In these products, listeria vanished faster at ambient (25°C) temperature than at chill (7°C), at pH 4.25 > pH 4.3 > pH 6.0, in fine emulsions > in coarse emulsions, and under anaerobic conditions > at aerobic conditions. From these experiments it was concluded that metabolic exhaustion is accelerated if more hurdles are present, and this might be caused by increasing energy demands to maintain internal homeostasis under stress conditions.

15.3.3 Stress Reactions

In relation to the preservation methods, the adaptation of pathogens to environmental and processing stresses constitutes a serious challenge for the food industry [27]. A limitation to the success of hurdle technology foods could be stress reactions of microorganisms. Some bacteria become more resistant (e.g. toward heat) or even more virulent under stress, since they generate stress shock proteins. The synthesis of protective stress shock proteins is induced by heat, pH, a_w, ethanol, etc., as well as by starvation. These responses of microorganisms under stress might hamper food preservation and could turn out to be problematic for the application of hurdle technology. On the other hand, the switching on of genes for the synthesis of stress shock proteins, which helps organisms to cope with stress situations, should become more difficult if different stresses are received at the same time. In order to counter different stresses simultaneously, the cell needs energy synthesis of several or at least much more protective stress shock proteins, which the microorganisms cannot deliver since they become metabolically exhausted [8]. Therefore, a multi-target preservation of foods could be the answer to avoid the synthesis of such stress shock proteins, which otherwise might jeopardize the microbial stability and safety of hurdle technology foods [26]. Nevertheless, further research in stress shock proteins, and the different mechanisms which cause their switch on or by which they could be avoided, seems warranted also in relation to hurdle technology foods.

In many instances, microorganisms are likely to be stressed or injured rather than killed in food processed by preservation technologies. The adaptation of microorganisms to stress during processing constitutes a potential hazard. Sub-lethal stress induces the expression of cell repair systems (Figure 15.5). For instance, nucleic acids are the primary target of ionizing radiations and UV light. When cells are exposed to UV, enzymatic photo repair and expression of excision-repair genes could restore DNA integrity. *E. coli* that survive UV exposure have an activated gene, i.e. a transcriptional regulator involved in multiple stress resistance [28]. Strains which survive high pressure have a similar type of enhanced gene-regulated resistance to both high pressure, light heat and cold shocks, and other stresses [27, 29, 30]. It was also observed that reversible DNA supercoiling protects cells against osmotic pressure [31]. However, microbial lethality by high pressure and pulsed electric fields is mainly attributed to changes in the membrane structure and functionality [32]. In addition, repetitive exposure of bacterial contaminants to the preservation process can transform highly resistant mutants [33]. Slow replication (i.e. under sub-optimal growth conditions) increases the time allowed for cell repair activity, and thus favors the recovery of sub-lethally injured microorganisms [34].

Kang et al. [35] studied the heat resistance of *Salmonella Enteritidis* in the presence of a salt and/or acid medium. *Salmonella* adapted to a single stress (acid or salt stress) and increased the heat resistance, whereas the combination of acid and salt stresses reduced heat tolerance; acid stress played a more critical role in mediating this effect as compared to salt concentration. Therefore, acid–salt combined preservation techniques and subsequent refrigeration may prevent *S. enteritidis* survival in heat-pasteurized food products caused by cross-protection of acid- or salt-adapted cells. Tola and Ramaswamy [36] measured the D and z values of *Bacillus licheniformis* as affected by different heating methods (conventional vs. ohmic), types of acidifying agent (citric vs malic acid), and pH levels (pH 4.5, 5.5, and 6.2) (Table 15.2). Temperature and pH showed a highly significant effect in destruction, while ohmic heating showed a marginally better rate as compared to conventional heating. The lowest D_{97} was 1.1 min at pH 4.5, when citric acid was used as an acidifying agent in ohmic heating. Types of acidifying agents did not show any effects on destruction.

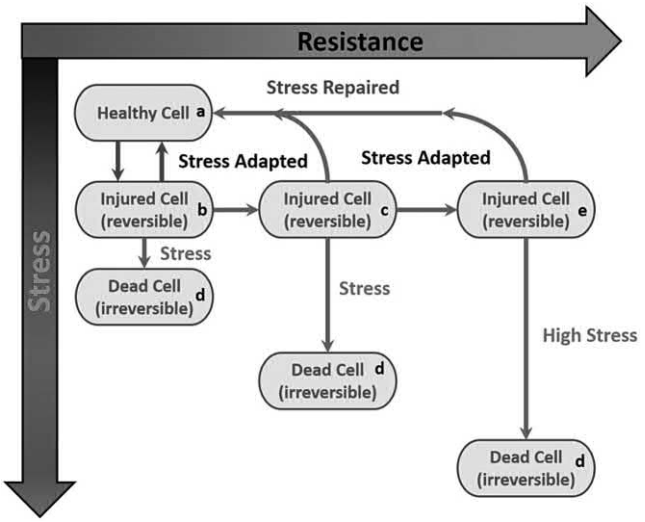

FIGURE 15.5 Microbial stress, injury, adaptation, and resistance to processing. (From Lado and Yousef [32].)

15.3.4 Multi-Target Preservation

The multi-target preservation of foods should be the ultimate goal for a gentle but most effective preservation of foods [8]. For foods preserved by hurdle technology, it has been suspected for some time that different hurdles in a food could not just have an additive effect on microbial stability, but act may synergistically [1]. Example 8 in Figure 15.3 illustrates this. A synergistic effect could occur if the hurdles in a food hit, at the same time, different targets (e.g. cell membrane, DNA, enzyme systems, pH, a_w, E_h) within the microbial cell, and thus disturb the homeostasis of the microorganisms present in several respects; then the repair of homeostasis as well as the switch on of stress shock proteins become more difficult [7]. Therefore, employing different hurdles simultaneously in the preservation of a particular food should have advantages, because optimal microbial stability could be achieved with an intelligent mix of gentle hurdles. In practical terms, this could mean that it is e.g. more effective to use different preservatives in small amounts in a food than only one preservative in larger amounts, because

TABLE 15.2
D and z Values of *Bacillus licheniformis* Spores in Carrot Juice Extract as Influenced by pH, Types of Acidifying Agent and Heating Methods

	Malic Acid			Citric Acid		
	pH 4.5	pH 5.5	pH 6.2	pH 4.5	pH 5.5	pH 6.2
T (°C)	D-value (min)			D-value (min)		
Conventional Heating						
87	7.2	9.5	11.2	7.2	8.4	9.1
92	4.8	6.6	7.5	4.2	5.1	5.6
97	1.6	1.9	2.2	1.2	1.5	2.0
z-value	12.6	14.5	14.2	12.6	13.3	15.3
Ohmic Heating						
87	5.8	7.9	9.8	5.4	6.5	9.2
92	3.8	5.1	5.8	3.3	4.2	5.9
97	1.5	1.6	1.7	1.1	1.4	1.6
z-value	17.0	14.7	13.2	16.3	15.1	13.0

Source: Tola and Ramaswamy [36].

different preservatives might hit different targets within the microbial cell, and thus act synergistically [14].

It is anticipated that the targets in microorganisms of different preservative factors (hurdles) for foods will be elucidated, and then the hurdles could be grouped in classes according to their targets within the microbial cells. A mild and effective preservation of foods, i.e. a synergistic effect of hurdles, is likely if the preservation measures include an intelligent selection and combination of hurdles taken from different "target classes" [7]. This approach seems not only valid for traditional food preservation procedures, but for modern processes (such as food irradiation, ultra-high pressure, mano-thermo-sonication, etc.) too. An example for a multi-target novel process is the application of nisin (which damages the cell membrane) in combination with lysozyme and citrate, which are then able to penetrate easily into the cell and disturb the homeostasis with different targets [26].

Food microbiologists could learn in this respect from pharmacologists because the mechanisms of action of biocides have been studied extensively in the medical field. At least 12 classes of biocides are already distinguished which often have more than one target within the microbial cell and multiple lesions in microorganisms are known. Often, the cell membrane is the primary target, becomes leaky, and unzips the organism, but biocides also impair the synthesis of enzymes, proteins, and DNA [37]. The "multi-drug attack" has proved successful in the medical field in fighting bacterial infections (e.g. tuberculosis) as well as viral infections (e.g. AIDS), and thus a "multi-target attack" on microorganisms should be a promising approach in food microbiology too [26].

Khan et al. [38] explored the potential of hurdle technology using combinations of novel technologies such as pulsed electric field, food irradiation, radiofrequency and microwave heating, Ohmic heating and high pressure, ozone and electrolyzed water treatment, and chemical preservatives and strategies for the food industry. However, they pointed out that further work is required to establish the proper combinations of different treatments in order to reduce the severities of unit processes and improve the overall properties and quality of food.

15.4 PREDICTION MODELS

Predictive microbiology attempts to describe the effects of environmental conditions on the growth, survival, and death of microorganisms. Hurdle technology needs both boundary and kinetic models. The boundary models provide the growth/no growth regions within a time-frame, while kinetic models provide the growth rate with time in the growth region [10]. Table 15.3 shows an example with four hurdles and four

TABLE 15.3
Examples of Growth/No-Growth Modeling and Prediction

a_w	pH	T_s (°C)	C_s	Growth/No-Growth	Rate
0.80	6.5	10	0.5	Growth	Needed
0.75	5.0	5	0.1	No-growth	0
0.95	4.1	25	1.1	No-growth	0
0.90	6.0	15	1.6	Growth	Needed

Note: These are arbitrary examples (not from experiments).
Specific type of bacteria or deteriorative reaction considered for a specific timeframe
a_w: water activity
pH: acidity
T_s: storage temperature (°C)
C_s: salt concentration (g/100 g sample)

possible scenarios indicating that the boundary is required first and then growth rates are needed.

15.4.1 Kinetic Model (Growth or Death Rate)

Bigelow [39] developed a mathematical model (i.e. kinetic model) to describe the logarithm nature of thermal death. Nevertheless, it was not until the 1980s that mathematical models predicting the behavior of microorganisms experienced a great development. A new field emerged in the food microbiology area: predictive microbiology. Most models used in the past in predictive microbiology have been inactivation models, to predict the death of microorganisms, in particular bacterial spores, caused by different treatments, such as heat, disinfectants, and radiation. It is only in the last two decades that kinetic models have been developed to predict the growth of food poisoning and recently also of spoilage microorganisms in foods. Different types of empirical growth models for microorganism are widely used. Kreyenschmidt and Ibald [40] reviewed different types of growth models. These are specific to the types of microorganism, physicochemical properties of food, and the hurdles used. Cornu et al. [41] developed a model for *Listeria monocytogenes* growth rates on cold-smoked salmon as a function of temperature, water-phase salt, and phenolic contents.

Davey et al. [42] developed the thermal inactivation rate (i.e. ln k) of *C. botulinum* as a linear function of 1/T, pH, and pH^2. Similarly, Cerf et al. [43] also developed the regression correlations of ln k as a function of 1/T, pH, pH^2, and a_w. The non-monotonous changes of pH and a_w make it more difficult to predict the effect of hurdles in combination. Velugoti et al. [44] generated growth data of *Salmonella* in sterile pork at various isothermal conditions, and dynamic (i.e. kinetic) models were developed within storage temperature (10–45°C).

Pujol et al. [45] developed a gamma-type model for the factors of storage temperature, pH, a_w, and acetic, lactic, and sorbic acids. It allowed the use of different combinations of hurdles to be quantified and compared to each other in terms of their inhibitory effect (i.e. iso-hurdle). They collected and analyzed existing data from industry, a public database, and the literature, after which a total of 650 growth rates were retained. Three steps were used to (i) build a predictive model based on existing literature data, (ii) build an experimental design focused on the iso-hurdle using the model output, and (iii) validate the model and the iso-hurdle rules with new data. The methodology used in the model allowed an assessment of the equivalence of inhibitory effects without intensive data generation; it could be applied to develop milder formulations which guarantee microbial safety and stability.

15.4.2 Boundary Models (Growth/No-Growth)

Increasing attention has been given to the development of models that allow the prediction of the probabilities of growth under different conditions and therefore allow predictions of growth/no growth boundaries or the growth/no growth interface [46]. In the 1970s the "probability model" (i.e. boundary model) for the prediction of germination of spores, population growth, survival, and toxin production within a specified period of time under defined conditions of storage and product composition emerged [47]. "Growth/no-growth" or "interface" modeling has progressed. Such boundary or probability models are of particular significance for ambient stable hurdle technology foods, and these should be applied to food-poisoning as well as to spoilage microorganisms. One of the first attempts at growth/no-growth (G/NG) modeling was developed by Pitt [48]. He related the boundary with the temperature and water activity limits for *Aspergillus* spp. growth by an empirical equation. McKellar and Lu [49] developed a probability-of-growth model for *Escherichia coli* 0157:H7 as a function of temperature, pH, sodium chloride, and acetic acid. The model predicted 99% correctly the growth/no growth of 1820 treatment combinations. Spoilage yeasts are often tolerant microorganisms in low-pH, low-a_w foods, and Lopez-Malo [50] developed a probability and boundary model. Carrasco et al. [47] reviewed the methodology of development of a probability or growth/no-growth (G/NG) model. The logistic regression approach has been widely adopted for probability and G/NG modeling. In general, probability models are devoted to data, which can be measured as "positive" or "negative." For example, if we consider the variable "detection of toxin," only two responses are possible: "detectable" or "not detectable." The responses can be coded as 1 (positive response) or 0 (negative response), or even better, if response replicates are obtained, a number between 0 and 1 can be given to the response, calculated as the average of the scores (0 or 1) of the replicates. The number obtained can be considered as the probability of occurrence of the phenomenon studied. This probability can be related to independent variables such as temperature or pH by some mathematical function using regression techniques. In logistic regression, the probability (P) is expressed as "logit":

$$logit\, P = \log\left(\frac{P}{1-P}\right) \tag{15.1}$$

Mertens et al. [51] developed a G/NG model by linear logistic regression, and *logit* was correlated as a function of a_w, pH, sodium chloride, and acetic acid. A simplified G/NG model was conceptually derived from the gamma model. Logit was related to the variables or factors in order to determine the boundary. Polese et al. [52] developed a G/NG boundary using the gamma function, and he suggested that limited number of fail dangerous predictions; thus he suggested that a simplified G/NG model could be used as a first estimate method when experimental data are missing or insufficient. In addition, a practical time-frame is usually used to generate the boundary data; thus the model ignores stability for longer periods. In general, extrapolation in prediction, especially in foods, is not straightforward because of the complexity of the food matrix [53].

In addition to gaining a fundamental understanding of the hurdles and their interactions, prediction models need to be available to apply hurdles in real food systems, and they are

key to determine the shelf life and safety. McMeekin et al. [54] reviewed the predictive microbiology and its applications. They revisited traditional predictive microbiology with an emphasis on temperature dependence and interpreted the temperature vs growth rate curve as comprising 11 regions, some already recognized. Their review envisioned a future of predictive microbiology in which models morph from empirical to mechanistic underpinned by microbial physiology and bioinformatics to grow into systems biology. They started by considering enzymes, which, being proteins, must be folded correctly to function, and considered what drives the folding process (i.e. is it thermodynamic or kinetic?) [55, 56]. The rate of protein folding is accelerated by molecular chaperonins, and it is essentially a spontaneous process [56]. In turn, this confirms the view of the 1972 Nobel Prize winner Anfinsen that the most probable conformation of a folded protein in any given environment is determined solely by its amino acid composition [57]. The work of Rothman and Shekman [58] highlighted the role of molecular chaperonins in accelerating rates of protein folding. The protein folding can be described by a mechanistic, thermodynamically based, and temperature-dependent model [59, 60]. The effect of other constraints on microbial growth, such as water activity and pH, can be modeled thermodynamically and can be extended to temperature dependence. These thermodynamic models could be applied not only in microbiology but also in protein stability, thermal biology, ecological theory, biogeochemistry, and extremophile biology and climate science. Finally, McMeekin et al. [54] envisioned that "the transition from empirical to mechanistic predictive models has its genesis, in part, in food microbiology."

15.5 CONCLUSION

The concept of single- and multi-hurdle effects in food preservation is now established through experimental evidence. Research is being conducted to improve the fundamental understanding, in areas such as homeostasis, metabolic exhaust, stress reactions, and microbial prediction models. It is important to develop both empirical and fundamental prediction models including wide ranges of multi-hurdles. Models could be an important tool for the food industry to develop safe food products with desired sensory and nutritional properties. Davidson et al. [61] stated "more research is needed on the effectiveness of antimicrobial combinations and antimicrobials in combination with physical methods (e.g. hurdle technology) that are effective against different groups of microorganisms. Combinations could well be the ideal antimicrobial for which everyone is searching."

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16 Hurdle Technology (Combined Methods) for Food Preservation: Applications

Lothar Leistner and Mohammad Shafiur Rahman

CONTENTS

16.1 INTRODUCTION

Foods based on combined preservation methods (hurdle technology) are prevalent in industrialized as well as in developing countries. Often hurdle technology is applied empirically without knowing much about the governing principles of the preservation of a particular food. However, with a better understanding of these principles and improved monitoring devices, the deliberate application of hurdle technology has advanced. Recently, Singh and Shalini [1] reviewed products developed using hurdle technology. Subsequently, examples of the application of combined preservation methods in industrialized and in developing countries were given.

16.2 ANALYZING THREE CATEGORIES OF FOODS

With regard to the application of hurdle technology, one might differentiate between intermediate-moisture foods, high-moisture foods, and integer foods, since they differ somewhat in the types of hurdles used or the mode of hurdle application. Therefore, these three groups are discussed separately.

16.2.1 Intermediate-Moisture Foods

Intermediate-moisture foods (IMFs) are in the water activity (a_w) range of 0.90–0.60, and thus water activity is their primary hurdle for securing microbial stability and safety. However, IMFs are often stabilized by additional hurdles, such as heating, preservatives, pH, redox potential, and competitive microflora [2]. These foods are easy to prepare and storable without refrigeration, thus they are cost and energy-efficient. Traditional IMFs based on meat, fish, fruits, and vegetables are common and much liked in different parts of the world because they are tasty, nutritious, and in general safe. On the other hand, newly developed, tailor-made IMFs (except for certain candy bars) have not achieved the expected breakthrough in human nutrition. Some reasons for this disappointing performance are the poor palatability of

most novel IMF due to the high concentration of humectants, and the need to introduce often high amounts of antimicrobial additives ("chemical overloading of foods"), which may cause health concerns and pose legal problems [3].

Traditional IMFs are today the prevalent food items in developing countries, even though practically none of the food manufacturers have the ability to measure water activity, and few recognize the relevance and significance of water activity for the preservation of their foods. Thus, the application of hurdle technology in the processing of IMF in developing countries is done empirically. Only recently have changes occurred. An outstanding example of these recent developments is the CYTED-D program (Science and Technology for Development) of Latin America, which was sponsored by Spain and to which Argentina, Brazil, Chile, Costa Rica, Cuba, Mexico, Nicaragua, Puerto Rico, Uruguay, and Venezuela have contributed. A project in this program, titled "Development of Intermediate Moisture Foods (IMF) Important to Ibero-America," had the objective to identify and evaluate foods of Latin America that are storable without refrigeration. This study comprised fruits, vegetables, and bakery products as well as foods derived from fish, milk, meat, and miscellaneous products. About 260 food items were approved to be microbiologically stable and safe at ambient temperatures. The properties (such as a_w, pH, preservatives, food composition) and the production technology for these products were measured and described [4, 5], and it was concluded that most of the approved products were intermediate-moisture foods. However, some had higher a_w values, sometimes as high as 0.97–0.98, and nevertheless were stable and safe without refrigeration, and it turned out that the stability of these high-moisture foods was caused by a combination of several, empirically applied hurdles. This observation was the starting point for Latin America to apply intentional hurdle technology [6] to high-moisture foods, especially to tropical and subtropical fruits, storable without refrigeration. In the opinion of Latin American scientists, the technological achievements of their region deserve a closer look, in particular by developing countries where refrigeration is scarce. Since IMFs are often not satisfactory from the sensory point of view and contain high levels of additives, the application of hurdle technology to stabilize high-moisture foods, which also need no refrigeration, seems to have great potential [5].

The Latin American CYTED-D study demonstrated a promising approach for improving the stability of foods in developing countries, which could be applied in other regions. Following this concept, first, the properties of already available, microbiological stable and safe food items should be thoroughly studied and described. Second, the preservative factors (hurdles) effective in these foods and thus the principles behind their microbial stability and safety must be elucidated. Third, if feasible, the preservation and quality of these foods should be improved by the intentional application of hurdle technology [3]. Using this concept, Tapia de Daza et al. [7] have identified the hurdles in food items studied within the CYTED-D program, and by critical evaluation of the hurdles that have been traditionally applied to certain foods, they assessed the microbial stability and safety of these products. It was demonstrated that similar hurdles are active in the same type of foods in different countries of Latin America. However, there were also surprising differences, which pointed to an over- or underprocessing of the same food in different countries of the same region [7]. This insight could lead to the avoidance of some nonessential preservatives or, on the other hand, to the improved stability, safety, and quality of some food items by fortification of certain hurdles.

Latin American researchers [5, 7] pointed out that reduced water activity was the main hurdle in IMFs as they have described within the CYTED-D project. However, in practically all of these products, additional hurdles were present that contributed considerably to stability and safety. This observation has confirmed the opinion expressed by Leistner and Rodel [2] that in most intermediate-moisture foods several hurdles are inherent. Tapia de Daza et al. [7] listed many traditional IMF of Latin America, derived from fruits, vegetables, meat, milk, fish, etc., in which several identified hurdles contributed to microbial stability and safety. Very often the reduced a_w was combined with a reduced pH. However, in some salted fish and shrimp the pH was more than 8.0, and thus in these foods, the elevated pH might contribute to the preservation. Many meat, fish, fruit, and dairy products contained additional preservatives (e.g., nitrite, smoke, benzoate, sorbate, sulfites, spices, Maillard products) and sometimes competitive microorganisms.

Maillard reaction products, which are generated during the caramelization of sweet condensed milk (dulce de leche), are probably an important hurdle for this Latin American food item. A special case concerns candied fruits common in this region. Their heavy sugar coating acts as a physical barrier (hurdle) against microbial contamination after the heat process, so that these foods are stable during storage in spite of the absence of preservatives and sometimes a rather high pH. Often a thermal treatment was used to inactivate heat-sensitive microorganisms during the manufacturing process and improve microbial stability, and a vacuum in sealed containers was achieved by hot filling of the products. Once the container is opened, the redox potential increases and then the microbial stability of these IMF against molds and yeasts must be secured by the presence of preservatives. Additional hurdles were employed in particular in those IMF, which had rather high a_w and/or pH values. In the IMF studied, it was exceptional if the a_w was apparently the only hurdle present, but in most IMF products three to five hurdles have been identified [7]. How many hurdles, besides a_w, are active in intermediate-moisture foods depends on the type of the product, however, it may be concluded that the microbial stability and safety of IMFs in general is based on a combination of several preservative factors.

It is obvious that a thorough study of traditional IMF using up-to-date methodology would be of benefit to developing countries. However, for industrialized countries such studies are rewarding because traditional products are an abundant source of innovative ideas that could be used in food design. For instance, we learned from traditional Chinese sausage (la chang) that a sausage could be preserved in the raw state even

without fermentation, or we realized that in traditional charqui of Brazil a fermentation takes place even at an a_w <0.90 if halophilic pediococci are involved. Heat inactivation of most pathogenic bacteria, including *Staphylococci*, is achieved in some Chinese IMF meats by just applying 50°C for several hours. Another interesting aspect of traditional IMF meats is the bactericidal effect of Maillard products toward food poisoning bacteria, because if these recontaminate the product after heating and drying they do not survive long, probably due to metabolic exhaustion. Apparently, the growth inhibition of xerotolerant molds on unpackaged Chinese IMF meats with a_w <0.69 is also supported by Maillard reaction products, which, therefore, are important hurdles for traditional IMF [8].

16.2.2 High-Moisture Foods

Intermediate-moisture foods are prevalent in developing countries, whereas in industrialized countries high-moisture foods (HMFs) are common, because they are often only minimally processed and due to their fresh-like properties and convenience are appealing to the consumer. However, since in HMFs the water activity is above 0.90, this hurdle is less prominent, and other hurdles have to secure microbial stability and safety of foods during storage. Therefore, HMFs are often chilled or frozen, and low-temperature storage is widely used. However, refrigeration is energy consuming and thus costly, and in case of temperature abuse, the stability and safety of the foods might be jeopardized. Therefore, besides the low-temperature hurdle for HMFs, additional hurdles (such as heating, pH, E_h, a_w, preservatives, and competitive flora), often applied in combination by means of hurdle technology, are significant. The hurdles are employed in HMF either empirically or now more often intentionally.

An example of the empirical use of hurdles in HMFs is Italian mortadella. This meat product is an emulsion-type sausage, which is traditional and very common in Italy, and might be even stored without refrigeration, as long as the sausage is uncut. Italian mortadella contains, due to a mild heat process (78°C core temperature), viable spores of bacteria. However, the growth of bacilli and clostridia is inhibited in genuine mortadella by a slightly decreased a_w (below 0.95), and this adjustment (by salt, sugar, milk powder, and drying) was done in the past without understanding the reasons. However, applying traditional recipes, the water activity has been surprisingly adjusted to the desired level <0.95, by hand-down experience [9–11]. In Germany, meat products preserved by a similar principle (pasteurization and a_w adjustment to <0.95 by drying of the sausage) are popular. These a_w shelf-stable products (SSP) are stable at ambient temperature.

Another example of a shelf-stable emulsion-type sausage of Europe is the Gelderse rookworst, in which intentional hurdle technology has been used for a number of years. A major hurdle of this product is the reduced pH, which is adjusted to 5.4–5.6 by the addition of 0.5% glucono-delta-lactone, and thus rookworst belongs to the group of pH-SSP. This sausage is stable for several weeks at ambient temperature, if vacuum packaged and reheated at 80°C for about one hour in the pouch. The heat treatment eliminates vegetative organisms, and bacterial spores are apparently not of much concern, as their numbers decrease during the heat process and surviving spores are inhibited by the pH and other hurdles present (e.g., nitrite). Gelderse rookworst is exported from the Netherlands in large quantities to Britain, and a pH >5.4 is acceptable from the sensory point of view. The binding of water and fat in rookworst is not problematic, in spite of the relatively low pH, if pork rinds and/or phosphates are added to the product [6, 9].

As mentioned before, IMFs are prevalent in developing countries. However, there is a trend also in developing countries to move gradually away from IMFs toward HMFs [12]. Because IMFs contain high amounts of humectants (such as sugar and/or salt) as well as many fungistatic preservatives (such as sorbate, benzoate, sulfite), they are undesirable from the sensory or nutritional point of view, and in addition they have a less appealing texture and appearance than HMFs. Therefore, efforts are being made to improve the quality of IMFs by decreasing sugar and salt addition, as well as by increasing the moisture content and a_w, without sacrificing the microbial stability and safety of the products if stored without refrigeration [9]. It might be expected that high-moisture, fresh-like foods that nevertheless are storable at ambient temperature, since they have been stabilized by intentional hurdle technology, will be on the increase in developing countries as soon as the application of advanced hurdle technology has been mastered [12].

16.2.3 Integer Foods

Whole or integer foods are not comminute and consist of large pieces of plant or animal tissue, nevertheless, their microbial stability and safety could be improved by the application of hurdle technology. Two approaches are common: (i) either the use of coatings that contain and maintain inhibitory substances in order to protect the surface of the foods against microbial deterioration or (ii) the dewatering and impregnation process, which consists of soaking foods in highly concentrated solutions of humectants or other food additions [9, 13].

An example of an application of a surface layer is pastirma, a traditional beef product of Muslim countries is storable for several months at ambient temperatures and is eaten in the raw state [14]. The stability and safety of pastirma are based on a reduced a_w (0.90–0.85), in combination with several additional preservative factors [12, 15]. The interior of this product is stabilized by dry curing of meat stripes (~5 cm thick) with salt and nitrate (which is reduced by bacteria to nitrite) and the removal of water by drying and pressing of the meat, as well as by the growth of lactic acid bacteria, which decrease the pH to about 5.5. These hurdles secure the inhibition of spoilage and pathogenic bacteria, including salmonellae, within the meat. The surface of pastirma is covered with an edible paste (3–5 mm thick) containing 35% freshly ground garlic and other spices (paprika, cumin, and mustard, as well as fenugreek as a binder). This surface coating prevents the growth of molds on the product during storage,

even at elevated humidity and temperature. Thus, at least five hurdles (a_w, nitrite, pH, competitive flora, garlic) are relevant to the preservation of pastirma.

Torres [16] studied the surface microbial stability of model foods by using coatings, which maintain preservatives and the desired low pH, and he confirmed that a low pH in the surface layer greatly improved the effectiveness of sorbic acid in this coating. Guilbert [17] used superficial edible layers for easily perishable tropical fruits and achieved preservation without affecting the integrity of food pieces. He pointed out that the formulation of edible films and coatings must include a component that can form an adequately cohesive and continuous matrix as well as the addition of a plasticizing agent to overcome brittleness. Specific agents (such as antimicrobials, antioxidants, organic acids, nutritional additives, flavors, and coloring) can be incorporated into edible films to obtain functional effects localized on the surface [18].

Edible coatings and osmotic dehydration represent two ways to apply hurdle technology to solid foods without affecting their structural integrity [18]. Osmotic dehydration is a dewatering and impregnation process that consists of soaking foods (such as fruits, vegetables, meat, cheese, and fish) in concentrated solutions of humectants (such as sucrose and sodium chloride) and is employed for solute transfer from a solution into the product [19]. With this process, also called "direct formulation," not only water-activity-lowering agents may be inserted into the food, but also preservatives and nutrients, as well as substances that control the pH, texture, and flavor, and this would build up positive hurdles that improve the stability as well as the quality of food products [18, 20]. As opposed to traditional soaking techniques (e.g., salting as used in cheese making, fish or meat curing, candying, and semi-candying), osmotic dehydration generally involves significant water removal (40–70 g of water is lost per 100 g of initial product) with limited and controlled solute incorporation (5–25 g of solute is gained per 100 g of initial product). Under typical operating conditions used for fruit and vegetables, mass transfer mainly occurs during the first two hours; thereafter, mass transfer rates become progressively slower until water loss stops, whereas solute gain continues to increase steadily. A soaking process does not generally produce stable products, but soaking has to be used as a preprocessing step before complementary processing steps, such as drying, freezing, pasteurization, canning, frying, and/or the addition of preservative agents [21].

16.3 APPLICATIONS IN INDUSTRIALIZED COUNTRIES

The deliberate and intelligent application of hurdle technology started in the mid-1970s in Germany [15] and was first used for the preservation of meat products [6]. Soon this concept was applied to a variety of food items in industrialized as well as in developing countries. In this review, some examples of advanced hurdle technology employed in industrialized countries for fermented, heated, or chilled foods, and for the design of healthful foods as well as in relation to the trend to less packaging of foods will be discussed.

16.3.1 Fermented Foods

In fermented foods—such as fermented sausages, raw hams, ripened cheeses, and pickled vegetables—a sequence of hurdles leads to a stable and safe product. For instance, in fermented sausages (salami) by the sequence of hurdles (i.e., preservative, E_h, pH, and a_w), food poisoning and the spoilage organisms are inhibited, and the desired competitive flora (lactic acid bacteria) is selected [9, 22–24] (Figure 16.1).

Important hurdles in the early stages of the ripening process of salami are nitrite and salt (preservative), which inhibit many of the bacteria in the batter. However, other bacteria are able to multiply, use up the oxygen, and thus cause the redox potential of the product to decrease. This in turn enhances the E_h hurdle, which inhibits aerobic organisms and favors the selection of lactic acid bacteria. They are the competitive flora and flourish by metabolizing the added sugars, which causes a decrease in pH value, i.e., an increase of the pH hurdle. In long-ripened salami, the nitrite is depleted and the lactic acid bacteria vanish, while the E_h and pH increase again, i.e., all these hurdles become weak during a longer ripening of salami. Only the water activity hurdle is strengthened with time and is then mainly responsible for the stability of long-ripened raw sausage [8, 22]. Since this sequence of hurdles and their effects has been revealed, the production of fermented sausages has become less empiric and more advanced, and this knowledge is used to achieve the required inhibition of *Clostridium botulinum*, *Listeria monocytogenes*, and *Staphylococcus aureus* as well as the inactivation of *Salmonella* spp. and verotoxins producing *Escherichia coli* in salami during fermentation and ripening. The sequence of hurdles that secures the microbial stability and safety of raw hams is also well known [25]. Probably in other fermented foods, such as ripened cheeses and pickled vegetables, a sequence of hurdles should be important for the proper fermentation process, and it would be challenging to elucidate them.

In Germany, fermented sausages are into two groups, i.e., quick-ripened products and slow-ripened products (Table 16.1). Quick-ripened products amount to about 80% of the production and the slow-ripened products to only 20%. In quick-ripened products, a_w is rather high, because these still contain much water and therefore are less expensive. However, to compensate for this high a_w, a low pH in such products is essential for microbial stability. On the other hand,

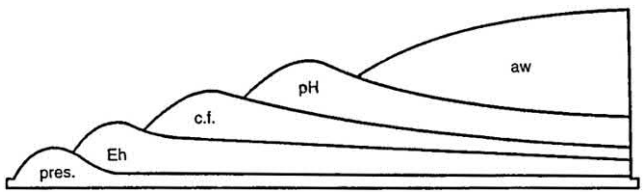

FIGURE 16.1 Sequence of hurdles occurring during the ripening and drying of fermented sausages (salami). Symbols have the following meaning: pres., addition of nitrite-curing-salt; E_h, decrease of redox potential; c.f., growth of competitive flora; pH, acidification; a_w, decrease of water activity during the drying process. (From Leistner [22].)

TABLE 16.1
Criteria of the Quick and Slow Ripened Fermented Sausages of Germany

Criterion	Quick-Ripened Products	Slow-Ripened Products
a_w	0.95–0.90	0.90–65
pH	4.8–5.2	5.4–6.0
Weeks	1–2	4–8
Production	8%	20%

slow-ripened products, which are more expensive due to the long drying period, have a low a_w, and therefore these products can afford a rather high pH, which makes them tastier. These differences between quick- and slow-ripened salami are mentioned here because they nicely illustrate that the hurdles in food are possibly interchangeable. Therefore, emphasis could be given to different hurdles to achieve microbial stability, with the consequences that the products have different features related to their sensory properties and price. Most of these sausages are air-dried or smoked, not ripened by growing molds. Lucke and Vogeley [26] developed slow-ripened traditional sausage by mincing meat, mixed with spices and minimal amounts of sugars, salt, and nitrate. It was stuffed and then allowed to ripen for a minimum of 6–8 weeks at temperatures below 15°C. In addition, surface mold growth during ripening was regularly removed or suppressed. This process required a minimum of investment and labor with a clean hygienic standard.

A feature peculiar to fermented sausages (and probably for ripened cheeses too) is their microstructure, which influences the desired ripening process as well as the survival of pathogenic bacteria in the product. Thus, the microstructure is an important hurdle for the stability of salami [3, 9]. Electron microscopy studies [27] revealed that the natural flora as well as added starter cultures were unevenly distributed in fermented sausages, but are arrested in little cavities of the product, i.e., the ripening flora can only grow in nests. These nests are 100–5000 µm apart, and thus large areas of the sausage must be influenced by metabolites (e.g., nitrate reductase, catalase, organic acids, and bacteriocins) accumulated in such nests or cavities. Thus, the entire fermentation of the product must be accomplished from small nests of desirable bacteria, and from these nests the pathogenic bacteria (e.g., salmonellae or listeria) must be inactivated and even their nests might be located in distant areas of the food matrix. Within each nest, the bacteria, either in pure or mixed cultures, are in keen competition for nutrients and impair each other with their metabolic products. In nests of mixed cultures, generally the lactic acid bacteria prevail due to their tolerance of low E_h, pH, and a_w. At the beginning of the sausage fermentation in these nests, the lactobacilli appear vigorous and metabolically active, whereas at the end of the ripening process the lactobacilli in their nests have degenerated and may have died [9, 27]. Small and equal distances between nests of desirable bacteria in the sausage matrix should be advantageous since this would foster the proper ripening process and the inactivation of pathogenic bacteria. The thorough mixing of the meat and fat particles of the sausage batter, before stuffing the sausage mix into casings, would bring about the desired more even distribution of bacteria in the sausage matrix. Moreover, if starter cultures are used, they should be added in a fashion that favors an even distribution, and this could be better achieved by using starter cultures not in a powdery state but in a liquid state [3, 9].

The microstructure is not only important for salami (and cheese) but for other foods too. In concentrated oil-in-water emulsions, the bacteria form small colonies, and in water-in-oil emulsions, the bacterial growths are confined to the water droplets, which might lose their integrity due to coalescence [28]. The impact of microstructure on microbial growth, survival, and death in foods has theoretical and practical implications. Certainly, under these circumstances predictive modeling of the behavior of microorganisms is difficult. On the other hand, it is possible to influence the number, size, and distance of microbial nests in such foods, and thus their safety, stability and quality, by the recipes of the product and the technology applied [3, 9]. The microstructure of foods is definitely an important hurdle for certain foods. Further studies on the behavior of submerged bacterial colonies in food matrices seem warranted [29].

16.3.2 Heated Foods

Heat-processed high-moisture foods based on hurdle technology, which are stable at ambient temperature, have been named shelf-stable products (SSPs) [6], and these offer the following advantages: the mild heat treatment (70°C–110°C) improves the sensory and nutritional properties of the food, and the lack of refrigeration simplifies distribution and saves energy during storage. SSPs are heated in sealed containers (casings, pouches, or cans), which avoid recontamination after processing. However, because of the mild heat treatment, these foods still contain viable spores of bacilli and clostridia, which are inhibited by an adjustment of a_w, pH, E_h, and, in the case of autoclaved sausages, by sublethal injury of the spores. At present four different types of SSP foods are distinguished (F-SSP, a_w-SSP, pH-SSP, and Combi-SSP), depending on their primary hurdles (Table 16.2), though additional hurdles foster the safety and stability of these products [3, 8, 9].

16.3.2.1 F-SSP

In one type, F-SSP [6, 8, 22], the sublethal damage of the spores is the primary hurdle, which is achieved by mild heat treatment. Examples are sausages with an adjusted a_w (Bologna-type sausage <0.97, liver and blood sausages <0.96) in polyvinylidene chloride (PVDC) casings, which are heated in counterpressure autoclaves to F_o >0.4. Such products are in large quantities and considerable variety since the mid-1980s in German supermarkets. These are stored unrefrigerated for several weeks and have caused no problems with regard to food poisoning or spoilage because guidelines for their processing have been suggested and followed [30]. F-SSPs, due to

TABLE 16.2
Different Shelf Stable Products (SSP) and Their Primary Hurdles*

F-SSP	Sublethal damage of bacterial spores
a_w-SSP	Slightly reduced water activity
pH-SSP	Slightly increased acidity
Combi-SSP	Combination of equal hurdles

* All are mildly heated foods, stable at ambient temperature, and nevertheless have fresh-product characteristics.

metabolic exhaustion of the microorganisms, even autosterilize during storage. For F-SSPs, casings are more advisable than cans, because during chilling of the cans after autoclaving, some water condensation may occur inside the lid, and if drops of water fall back on the surface of the sausage mix, locally the critical a_w increases and thus growth of clostridia may start in this portion of the product. If autoclaved sausages fill the casings tightly, water condensation inside the container cannot occur, and therefore F-SSP in casings are more stable than in cans with headspace [31].

16.3.2.2 a_w-SSP

The stability of another type, a_w-SSP [6, 8, 9, 11, 22], is primarily caused by the reduction of water activity below 0.95, and guidelines for their processing have been suggested [22]. Examples of traditional a_w-SSP meats are Italian mortadella and German brühdauerwurst, however, a large variety of such meat products is now on the market, and most of them are snack items. The shelf life of a_w-SSP at ambient temperature is even better than with fermented sausages since in a_w-SSP, due to the heat treatment (internal temperature >75°C), lipases are inactivated and thus these products are less prone to become rancid.

16.3.2.3 pH-SSP

In the third type, pH-SSP [6, 8, 22], increased acidity is the primary hurdle. This principle is applied in Gelderse rookworst, a shelf-stable meat product. Another traditional meat product of the pH-SSP type is brawn, and these jelly sausages are adjusted to an appropriate pH by the addition of acetic acid. Such products are, for example, composed of a brine (pH <4.8) made of water, gelatine, salt, sugar, agar-agar (2%), and spice, and a solid phase, made of bologna-type sausage in cubes with a_w of <0.98. Both components are mixed (two parts brine and three parts meat), filled in casings and heated to an internal temperature of >72°C but not higher than 80°C. If the product is in equilibrium, it should have a final pH <5.2, and then it is storable for several days at ambient temperatures. Outside the meat field, pH-SSPs are common as heat pasteurized fruit and vegetable preserves with pH <4.5, which are bacteriologically stable and safe, in spite of mild heat treatment. In such products, vegetative microorganisms are inactivated by heat, and the multiplication of surviving bacilli and clostridia is inhibited by the low pH. Since bacterial spores are able to germinate at lower pH levels than vegetative bacilli and clostridia are able to multiply, in pH-SSPs, as in F-SSPs and a_w-SSPs, the number of spores tends to decrease during storage due to metabolic exhaustion of microorganisms. On the other hand, while the heat resistance of bacteria and their spores is enhanced with decreasing a_w, it is diminished with decreasing pH. Thus, pH-SSPs need less heat treatment for inactivation of microorganisms than do a_w-SSP.

16.3.2.4 Combi-SSP

In the fourth type, Combi-SSP [8, 9, 32], a combination of rather equal hurdles is applied, each of which adds a little on an imaginary balance [8], which should swing from the unstable to the stable state of the product. Even small enhancements of the individual hurdles in a food in summation have a definite effect on the microbial stability of a product. For instance, for the stability and safety of a food it is of significance whether the F_o is 0.3 or 0.4, the a_w is 0.975 or 0.970, the pH is 6.5 or 6.3, and the E_h value is somewhat higher or lower. Every small improvement or reinforcement of a hurdle brings some weight to the balance, and the sum of these weights determines whether a food is microbiologically unstable, uncertain, or stable (Figure 16.2). In other words, all little steps in the direction of stability decide whether the balance swings from an unstable into a stable state of the product [8]. It is followed in designing of Bologna-type sausages as Combi-SSP. Different types of brühwurst (wieners, bockwurst, fleischwurst, fleischkäse, etc.) were developed, which proved microbiologically stable and safe at least for one week at 30°C. The initial spore load of the sausage mix is low because spice extracts instead of natural spices are used, nitrite (100 ppm) with curing salt must be added, and these products are heated to a core temperature >72°C, and are adjusted to an a_w and pH of <0.965 and <5.7, respectively. These products are repasteurized after vacuum packaging for 45–60 min (depending on the diameter of the products) at 82–85°C [33]. Combi-SSPs offer opportunities for many food items, however, they require strict rules for food design and process control. The Combi-SSP concept is not only applicable to meat products but to other foods too.

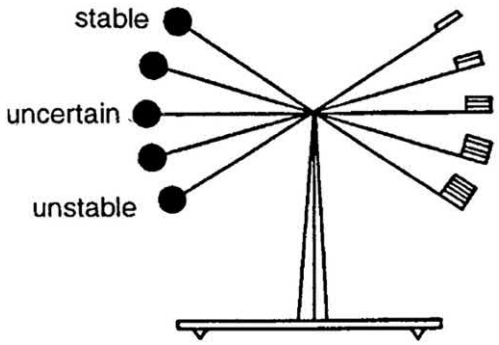

FIGURE 16.2 The balance should illustrate that even small enhancements of different hurdles could in summation bring about a substantial improvement of the microbial stability of a food. (From Leistner [8, 48].)

For instance, an Italian pasta product (tortellini) was stabilized by using as hurdles a water activity reduction and a mild heat treatment, as well as modified atmosphere or ethanol vapor during storage, combined with moderate chilling temperatures [34, 35]. Another example is paneer, a dairy product of India, which was developed as a Combi-SSP [36, 37].

The Federal Centre for Meat Research in Germany demonstrated the efficiency of the application of hurdle technology in an extensive study (supported by the Medical Corps of the German Army) on 75 meat products with fresh-product characteristics, which nevertheless were storable without refrigeration [9, 32, 33]. In this study eight categories of meat products were selected and optimized, one represented fermented sausages and most of the others were F-SSPs, a_w-SSPs, and Combi-SSPs (Table 16.3). Since these meats must be suitable for army rations during military exercises, they had to be stable and safe at least for 6 days at 30°C, and they should be tasty and nutritious. In this situation, a linkage between hurdle technology and HACCP are introduced [9, 38]. In the manufacturing plants processing the recommended meats for the army, no microbiological tests must be carried out, however, time, temperature, pH, and a_w must be strictly controlled. These measurements should be done on-line, or at least close to the line. Fortunately, a precise instrument for this purpose became available [39], which allows a_w determinations of meat products within 10–20 minutes. This army study might be used for other occasions, in which hurdle technology and HACCP should be linked [32].

16.3.3 Chilled Foods

The results of hurdle technology are most obvious in high-moisture foods, which become shelf-stable at ambient temperature due to an intelligent application of combined methods for preservation. However, the use of hurdle technology is appropriate for chilled foods too, because in case of temperature abuse, which easily might happen during food distribution, the stability and safety of chilled foods could break down, especially if low-temperature storage is the only hurdle. Therefore, it is reasonable to incorporate into chilled foods (e.g., sous vide dishes) some additional hurdles, which would act as a backup in case of temperature abuse. This type of safety precaution for chilled foods might be called "invisible technology" [9, 40], and this implies additional hurdles act as safeguards in chilled foods and ensure that they remain microbiologically stable and safe during storage in retail outlets as well as in the home.

However, for many chilled foods, additional hurdles are already routinely used. This is particularly true for refrigerated foods for which modified atmosphere packaging (MAP) is employed. Raw and minimally processed vegetables are amongst the most popular ready-to-eat foods that are stored chilled in MAP [41, 42]. In Europe, MAP is mainly applied to salads, potatoes, carrots, and cabbage, whereas in the United States the fresh-cut produce market is stocked mainly with salads, paprika, onions, cabbage, mushrooms, endive, and spinach. MAP is intended to suppress microbial growth, retard respiration, ripening and aging of vegetables, and to inhibit oxidative reactions requiring oxygen. In combination with appropriate chilling, shelf life of foods under MAP can be up to 5–7 days. Immediately before sealing, a gas of defined composition is introduced in the packages that are generally stored at 4–7°C. Typically, the gas composition in MAP for fresh and minimally processed vegetables is 2–5% oxygen and 5–10% carbon dioxide. The modified composition of the gas atmosphere in MAP systems has a marked impact on the growth of spoilage microorganisms as well as on pathogens [43–45]. Minimally processed vegetables are not sterile and numerous genera of spoilage bacteria, yeasts, and molds are frequently encountered, and some cold-tolerant pathogens (*E. coli* 0157:H7, *Bacillus cereus*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *L. monocytogenes*) are hazards for MAP produce and all have been reported to occur on vegetables [46]. Even the CO_2 in MAP does not inhibit the pathogens directly; the competitive flora that grows in MAP at reduced oxygen and increased carbon dioxide might act as a hurdle and thus suppress the pathogens mentioned [43]. However, occasionally *L. monocytogenes* might under certain conditions in MAP systems proliferate to hazardous levels. Additional preservative factors (hurdles), e.g., *Enterococcus mundtii* as protective culture, may be integrated into the MAP system to ensure a safety better than what is possible with current standards [47].

TABLE 16.3
Categories of Meat Products on the German Market That Are Fresh-Like and Storable without Refrigeration due to the Application of Hurdle Technology

1. Quick-ripened fermented sausage
2. Mini-salami (two different technologies)
3. Brühwurst and liver sausage as F-SSP
4. Dried brühwurst as a_w-SSP
5. Repasteurized brühwurst as a_w-SSP
6. Brawns and brühwurst as pH-SSP
7. Items of brühwurst as Combi-SSP
8. Brühwurst in autoclaved flat pouches

Sources: Hechelmann et al. [32, 33]; Leistner [9]; Leistner and Hechelmann [32].

16.3.4 Healthful Foods

Consumers demand healthy foods, which shall contain less fat, salt (sodium), and cholesterol. Because of this trend, muscle foods, derived from red meat, poultry, or fish are modified. However, less salt or fat as well as their substitutes and replacers might diminish the microbial stability and safety of foods, since important hurdles change. Compensation of these deficits can be achieved by the application of intelligent hurdle technology [48, 49].

Several substitutes and/or enhancers for sodium chloride (i.e., various other salts and hydrolysates) and >100 fat replacers (i.e., fat mimetic systems or synthetic fat replacers) are available today and of potential use for modified muscle foods. However, the microbial consequences of their use are generally unknown. Increased water activity is probably of major importance for the stability and safety of low-fat and/or low-salt muscle, and acidity might be unfavorable too since low-salt muscle foods have a slightly higher pH compared with the normal products. If fat is replaced by proteins, the pH and/or the buffering capacity might increase, and thus the microbial stability and safety of these products would decrease. Added preservatives might be diluted, and thus render them less effective. This is due to the increased water-holding capacity caused by fat replacers. Furthermore, the redox potential (E_h) might change, because proteinaceous fat replacers might increase the E_h-buffering capacity of a food, whereas foods with a high water content should have a low E_h-capacity. The microstructure of some foods (e.g., emulsions, fermented products) is significant for their microbial stability and safety. This microstructure could change by the addition of some fat replacers, however, this effect has not yet been investigated. More data are needed on the impact of different replacers for fat and substitutes for salt (sodium), as well as of relevant combinations and on the important preservative factors (hurdles) of modified foods. The obtained quantitative data should be the basis for the microbial stability management of novel low-fat and/or low-salt muscle foods and would make the design by intelligent hurdle technology feasible.

To keep low-fat and/or low-salt muscle foods microbiologically stable and safe, the refrigeration during storage of these products must be perfect. Since this cannot always be guaranteed, the weak hurdles in modified products should be compensated by alternative preservative factors. In the design, processing, and marketing of low-fat and/or low-salt muscle foods, microbiologists should take an active part and close cooperation with technologists will prove fruitful. Hitherto in the design and production of low-fat and/or low-salt muscle foods, the nutritional aspects have been more emphasized than the microbial aspects. However, microbiology must not be neglected, because these foods will only be continuously accepted by the consumer if they cause no food poisoning and do not spoil easily [49, 50].

16.3.5 Packaging of Foods

Packaging is an important hurdle for most foods since it supports microbial stability and safety as well as the sensory quality of the products. Because of the various types of hurdle technology foods known, the appropriate packaging materials and procedures must be selected and applied with respect to the individual properties of the food items [40, 48].

Industrialized countries have the tendency to overpackage their foods, whereas in developing countries there is a lack of knowledge and of simple packaging materials for foods. Therefore, further exploration into the application of easy-to-\use, cheap, environmentally friendly, and efficient packaging for hurdle technology foods of developing countries should be a challenging and even a lucrative approach [48].

Packaging in industrialized countries is sophisticated, because absorbers, scavengers, scrubbers, and getters or emitters for gas components as well as desiccants, antimicrobial packaging material, tuned infrared films, edible food coatings and films, time–temperature integrator tags, microwave doneness indicators, etc. are employed for food items [51, 52]. These procedures and devices might be summarized under the term "active packaging." Japan was in many respects and for several decades the world champion in food packaging, and recent developments in active or smart packaging frequently originated in this country. However, since the mid-1980s several Japanese packaging experts have cast doubt on these developments and questioned whether such strategies in food packaging should be pursued in the future. According to the opinions of Japanese packaging experts, which were conveyed by Ono [53], five generations of food packaging are distinguishable. First came the function of "containing," i.e., since prehistorical times people have used vessels, pots, baskets, etc. as containers for foods. The second generation of packaging had the function of "transporting," i.e., wrappings, bottles, barrels, etc., which were used for food transport and distribution. The third generation provided an extended shelf life to foods by hermetically "sealing" the packages, and this made canning, vacuum, and modified atmosphere as well as aseptic packaging possible. In the fourth generation the packaging would "create quality" during distribution of the food, i.e., the packaging itself has a processing function, in addition to the common processing of foods. Therefore, such systems are called smart or active or functional packaging, and they are sophisticated but costly and wasteful too. In addition they create environmental problems, which in a densely populated country like Japan have already had severe consequences. Therefore, recycling and/or reduced packaging have become necessary goals. Japan is taken aim with the fifth generation of packaging at "less packaging" of foods, and this generation might be called the "climax of packaging or its despair" [53]. In the fifth generation, the shelf life and quality of foods should not be provided by the packaging but by the food itself or by the mode of distribution (Table 16.4).

TABLE 16.4
Generations of Food Packaging Suggested in Japan

First: Containing	People used packaging (i.e., pots, baskets) since pre-historical days for containing foodstuffs
Second: Transporting	Packaging is used to transport foods in bottles, barrels, containers, cartons, etc.
Third: Sealing	Possible canning of foods, vacuum, modified atmosphere, and aseptic packaging possible
Fourth: Create quality	Active packaging improves the food through scavengers, absorbers, getters, emitters, etc.
Fifth: Less packaging	Just-in-time distribution of foods or advanced hurdle technology foods provide the shelf life

Sources: Ono [53, 54].

In the opinion of Japanese experts, future packaging should provide only information (name and picture of product, nutritional and shelf life data, etc.), and some convenience to the consumer. The required shelf life of the food shall not come from the packaging, but shall be secured either by (i) aseptic packaging combined with just-in-time delivery systems, or by (ii) development of stable and safe hurdle technology foods.

Aseptic (ultraclean) packaging of food is perfectly executed in Japan because factory workers carry out orders with discipline and diligence. If due to ultraclean packaging only few microorganisms are "at the start" in the food, only a few hurdles of low intensity are needed to inhibit microbial growth, and thus ultraclean packaging is an important component of hurdle technology [3, 8]. Ultraclean packaged foods need only simple packaging if a quick delivery at low temperature is guaranteed. In Japan, such foods are transported close to freezing point, and just-in-time distribution systems have been widely introduced because they provide the needed shelf life at low costs due to reduced inventory at the point of sale [53]. Delivery is often done three times a day, and this just-in-time delivery proved successful and is expected to increase rapidly. However, the frequency of the transportation needed could also be a burden on the environment.

Another method to avoid elaborate packaging is to design foods that are stable, safe, and of a superior sensory quality in spite of minimal packaging. In this respect, Japanese authors discussed initially the promotion of intermediate-moisture foods [53]. However, after getting acquainted with hurdle technology applied to high-moisture foods, the latter concept became a promising option for achieving the fifth generation of food packaging [54].

16.4 APPLICATIONS IN DEVELOPING COUNTRIES

There exists an abundant variety of preserved foods in developing countries, because the gap between harvest peaks has to be bridged, and the taste as well as the nutritional properties of foods might improve due to preservation. Most of the preserved foods in developing countries, which preferably must be storable without refrigeration, are based on empiric use of hurdle technology. However, several foods have already been optimized by the intentional application of hurdles. The state-of-the-art in the use of hurdle technology in some countries of Latin America, Asia, and Africa has been presented in a comprehensive review [12].

16.4.1 Fruits of Latin America

During the last decade a novel process for the preservation of high-moisture fruit products (HMFP: $a_w > 0.93$) has been developed in seven Latin American countries (Argentina, Costa Rica, Cuba, Mexico, Nicaragua, Puerto Rico, and Venezuela), and has been applied to peach halves, pineapple slices, mango slices and purée, papaya slices, chicozapote slices, banana puree, plum, passion fruit, and tamarind, as well as whole figs, strawberries, and pomalaca [55, 56]. The new technologies were based on the combination of a mild heat treatment (blanching for 1–3 min with saturated steam), slight reduction in water activity (to 0.98–0.93, by addition of glucose or sucrose), lowering of pH (to 4.1–3.0, by addition of citric or phosphoric acids), and the addition of antimicrobials (potassium sorbate or sodium benzoate, and sodium sulfite or sodium bisulfite) in moderate amounts to the syrup of the products (Table 16.5). During storage of HMFP the sorbate and in particular the sulfite levels decreased, whereas the a_w fell (the a_w hurdle increased) due to the hydrolysis of sucrose [55]. Thus, "combined methods technology" (hurdle technology) was applied in these novel processes [55–59]. The minimal processes proved inexpensive, energy-efficient, simple to carry out (little capital investment), and were satisfactory to preserve fruits *in situ*. The resulting fresh-like products were still scored highly by a 30–50 member consumer panel after 3 months of storage at 35°C for taste, flavor, color, and especially for texture, which is often problematic for canned fruits. Thus, according to Latin American researchers [55, 56, 58, 60, 61], the combined methods applied allow storage of fruits, without losses between seasonal harvest peaks; for direct domestic consumption; or for further processing to confectionery, bakery goods, and dairy products; or for preserves, jams, and jellies. Fruit pieces can also be utilized as ingredients in salads, barbecues, pizzas, yogurt, and fruit drink formulations [56, 61]. Moreover, these novel HMFPs could open new possibilities for export markets.

The high-moisture fruit products stabilized by hurdle technology proved shelf stable and safe for at least 3–8 months storage at 25–35°C. Due to the blanching process, the initial microbial counts were substantially reduced, and during the storage of the stabilized HMFPs, the number of surviving bacteria, yeasts, and molds further decreased, often below detection limits [55, 56, 58–62]. Banana puree challenged with yeasts, molds, clostridia, and bacilli, known to spoil fruits, and stored at ambient temperatures for 120 days remained stable if proper hurdles were applied (mild heat treatment,

TABLE 16.5
Typical Process Used for the Preservation of High-Moisture Fruit Products (HMFP), as Developed in Latin America

Hurdle	Intensity
1. Heat treatment[a]	Saturated steam for 1–3 min
2. Water activity[b]	a_w, reduction to 0.98–0.93
3. Acidification[c]	pH, adjustment to 4.1–3.0
4. Preservative (I)	1000 ppm sorbate or benzoate
5. Preservative (II)	150 ppm sulfite or bisulfite

Source: Alzamora et al. [55, 56]; Argaiz et al. [60]; Tapia de Daza et al. [61]

[a] Dependent on the size and type of fruit.

[b] Adjusted with sucrose, glucose, maltodextrine, etc.

[c] Adjusted, if necessary, with phosphoric or citric acid

adjustment of a_w to 0.97 and pH to 3.4, addition of 100 ppm potassium sorbate, 400 ppm sodium bisulfite, and 250 ppm ascorbic acid). The inoculated microorganisms declined and often vanished below the detection limit [57]. These favorable microbiological results obtained with HMFP are probably due to "metabolic exhaustion" of the microorganisms present in the stabilized products. Since HMFP during storage at ambient temperatures become apparently sterile, pathogenic and toxigenic microorganisms are not likely to be a hazard for these foods [12].

Alzamora et al. [56] expressed the opinion that HMFP technologies as developed in Latin America could attract much attention in many developing countries because they are easy to implement and considerably improve the quality of stored fruits. They even believe that the usefulness of combined methods preservation (hurdle technology) for HMFP may give rise to an "explosion" of research on minimally processed fruits, and the application of this innovative process by the food industry [55]. Latin American advances in fruit preservation are impressive and have recently been confirmed by Indian researchers, who concluded that "hurdle technology is seen as a promising technique for the preservation of fresh fruits and vegetables" [63]. However, the preservation of HMFP must certainly be based on guidelines for good manufacturing practice (GMP) in order to be successful under industrial or even artisan conditions [64]. For instance, the reuse of syrup may become a risk in relation to a build-up of the spoilage flora (e.g., *Zygosaccharomyces bailii*, which could be sorbate-resistant), and therefore the reuse of syrup in HMFP processes should only be recommended after pasteurization.

Preciado-Iniga et al. [65] developed a stable tamarillo sweet product by applying hurdle technology. The hurdles were blanching (boiling water 93°C for 12 min), low pH, reduction of water activity, sodium benzoate and potassium sorbate (1:1) addition, and stored at 4°C, with oxygen and light protection. The product was stable up to 56 days considering permitted limits of aerobic mesophilic bacteria (<50 CFU/g) and molds and yeasts (<10 CFU/g), without changes in anthocyanin, phenolic, antioxidant capacity, and color. However, the unblanched tamarillo was microbial stable up to 42 days.

16.4.2 Fruits and Vegetables in India

Sinha et al. [66] developed microbial and sensory stability of fresh cauliflower for 180 days using hurdle technology. They followed five steps: (i) optimize the blanching process (temperature: 98–100°C, 30–60 s), (ii) potassium metabisulfite dipping treatment (0.25% for 10 min), (iii) steep in preservatives solution (i.e., six treatments with a combination of salt, potassium metabisulfite, sodium benzoate, and citric acid), (iv) aseptic packaging, and (v) chilled storage (temperature: 30–37°C and 5–7°C). The following treatments showed microbiologically safe products until 180 days with the highest sensory qualities. These are (i) steeping 8% salt, 0.3% citric acid, 300 ppm potassium metabisulfite, 300 ppm sodium sorbate, storage 30–37°C; (ii) steeping 10% salt, 400 ppm citric acid, 500 ppm metabisulfite, 100 ppm sodium benzoate, storage 5–7°C; and (ii) steeping 8% salt, 0.3% citric acid, 300 ppm potassium metabisulfite, 300 ppm sodium sorbate, storage 5–7°C.

16.4.3 Meats of China

Even though the per capita consumption of meat, compared to Western countries, is rather low in China, due to the huge number of people living in China this country has the largest meat consumption worldwide. Pork is the preferred meat in China, however, beef, water buffalo, sheep, poultry, and rabbit are also used as raw materials [67]. Only about 15% of the available meat of China is processed [68]; however, this is still a large amount and meat products are an important and precious part of the diet. Two categories of meat products exist in China side by side: Chinese meats and Western meats. Of the Western meat products, recently autoclaved emulsion-type sausages have gained ground in China. Their technology has been derived from the German F-SSP. However, the a_w of the Chinese products is not adjusted, and therefore they must receive a severe heat treatment (F_o >5.0), which is not beneficial for the taste but results in shelf-stable products. On the other hand, the recipes of traditional Chinese meat products date back centuries, and their microbial stability and safety are based on combined preservative factors empirically applied. Studies are in progress to identify the hurdles inherent to these traditional meats [69]. The concept of hurdle technology was introduced to China and work related to the application of hurdle technology to meat products is being carried out in several Chinese research institutes [70–74].

Traditional Chinese meat products are quite simple to prepare without expensive equipment, have a typical flavor, are ready-to-eat, and storable for an extended time without refrigeration. The traditional meats of China listed in Table 16.6 are all intermediate-moisture foods, and this implies that they might be too salty or too sweet, too tough, and too dark due to the formation of such Maillard reaction products. The water

TABLE 16.6
Traditional Chinese Meat Products*

Cured meats (yan la)	Chinese bacon, la rou
	Pressed duck, ban ya
	Silk rabbit, cha si tu
	Cured chicken, yuan bao ji
Dried meat (rou gan)	Dried meat, rou gan
	Sweet meat, rou pu
	Meat floss, rou
Sausage (la chang)	Guangdong la chang
	Sichuan la Chang
Raw ham (ho tui)	Yü nam ho tui
	Jin hua ho tui

Sources: Leistner [12]; Wang and Leistner [69].

* All these meat products are intermediate-moisture foods and are storable without refrigeration.

activity is the primary hurdle in these meats, but if additional hurdles are strengthened, then the a_w might be raised and this often improves the sensory quality of the products. However, the microbial stability and safety of the meats must not be jeopardized by an increased a_w. Therefore, intentional and intelligent hurdle technology is increasingly applied.

Rou gan is a typical dried meat product of China, prepared mainly from beef, using a technology that has not been changed for hundreds of years, but improvements are possible and desirable. Consumers now prefer products with a softer texture, lighter color, and less sugar. Shafu is a modified dried meat that fulfills these expectations. The a_w of rou gan is in the range of 0.60–0.69, whereas shafu has an a_w of 0.74–0.76, because its moisture is higher and the sugar content lower. Additional hurdles in shafu are nitrite and ascorbic acid, which improve the color and delay rancidity, whereas rou gan is cured with nitrate only. Furthermore, the microbial stability of shafu is improved by the selection of raw material with low microbial load, low temperature during curing but relative high temperature and shorter times during heating, minimizing of recontamination after the heat process as well as vacuum packaging of the final product in order to inhibit mold growth and to delay rancidity. Therefore, shafu is microbiologically stable and safe for several months at ambient temperatures, and thus has the same shelf life as the traditional rou gan [68]. Both products have low residual levels of nitrite and nitrate, contain few microorganisms including in general no pathogenic or toxigenic bacteria, and thus are safe meats.

A similar approach was chosen to improve the quality of Islamic dried beef, which is also storable for several months without refrigeration. The traditional product is just salted and dried, and therefore is very salty and becomes easily rancid. The modified product contains less salt, is cured with nitrate to improve color, and some ascorbic acid is added to delay rancidity. Furthermore, vacuum packaging is applied to reduce oxidation and to avoid mold growth [75]. More examples of the application of hurdle technology to Chinese meat products are available in a review article [12].

Finally, the stabilization of the Chinese sausage, which was achieved in cooperation with Taiwan [76], will be mentioned. Chinese sausage is highly esteemed by oriental consumers and differs from the fermented sausages common in Western countries. It is processed raw, with little fermentation. Several types of la chang (also called lup cheong) are known, e.g., the Sichuan type is spicier and the sweeter Cantonese, whereas the Taiwanese la chang is softer. In mainland China, finished products of la chang have the following properties: a_w 0.85–0.70, pH 5.9–5.7, NaCl 3–5%, sugar 4–20%, total count $<10^6$ /g, shelf life 2–3 months without refrigeration and if vacuum packaged 4–5 months (Table 16.7). To the coarsely ground meat and pork fat, sugar, salt, soya sauce, liquor, spices, nitrate (or nitrite), and sometimes sodium ascorbate, are added. The mix is stuffed into casings with small diameter, and the sausages are dried fast over charcoal at 45–60°C to a_w <0.92, and then further at ambient temperature to a_w <0.80, in order to avoid an increase of lactic acid bacteria counts, since most Chinese dislike sour meats. Thus, la chang is a typical meat product preserved by combined factors. In general, before consumption la chang is sliced and heated in rice or vegetable dishes [5, 22, 72, 76].

TABLE 16.7
Examples of Meat and Paneer Based on Hurdle Technology

Product	Hurdles	Shelf Life
La chang (meat) (China)	a_w: 0.85–0.70 pH: 5.9–5.7 NaCl: 3–5% Sugar: 4–20% Refrigeration: no Total count < 10^6/g	2–3 months
La chang (meat) (China)	a_w: 0.85–0.70 pH: 5.9–5.7 NaCl: 3–5% Sugar: 4–20% Packaged: vacuum Refrigeration: no Total count <10^6/g	4–5 months
Fresh paneer (India)	Storage: 35°C	1–2 days
Paneer (India)	a_w: 0.97 pH: 5.0 F_o: 0.8 min Storage: 35°C	Several weeks
Paneer in gravy (India)	a_w: 0.95 pH: 5.0 F_o: 0.8 min Potassium sorbate: 0.1%	2 weeks (storage: 45°C) 1 month (storage: 30°C) 3 months (storage: 15°C)

Sources: Kuo et al. [76]; Rao [36]; Rao et al. [37]; Rao and Patil [85].

The Taiwanese variety of Chinese sausage contains more moisture and therefore can have a_w as high as 0.94. This improves the sensory properties since the products are softer, but decreases their microbial stability and safety, because in such products lactic acid bacteria still grow and lead to a sour taste, and *S. aureus* might cause food poisoning. After the addition of 3.5% sodium lactate and 1.0% sodium acetate, the Taiwanese sausage remains tasty but is rendered microbiologically stable and safe, even if stored for several weeks without refrigeration. These additives reduce a_w of the product but also have some antimicrobial effects [76]. Challenge tests using inoculum of *S. aureus*, *L. monocytogenes*, or *Salmonella* spp. confirmed that Taiwanese sausage, which was modified by intentional hurdle technology, was stable and safe [76]. Furthermore, they demonstrated that in this product the number of pathogens decreased faster during storage at 25°C than at 10°C. This was probably due to a faster metabolic exhaustion of the pathogens at the higher storage temperature.

16.4.4 Dairy Products of India

The application of hurdle technology to traditional and modified Indian food products has been applied [37, 63, 77, 78].

Paneer is a traditional, cottage-cheese type product fried in cubes with oil and onions, to which a sauce containing salt, spices, and often tomatoes is added. This food is frequently consumed and much liked in the northern provinces of India, because of its nutritive value and characteristic taste. However, paneer spoils bacteriologically within 1–2 days at room temperature (which in India can reach 35°C), and this is a strong drawback for its industrial production. Sterilized paneer in cans has severe sensory limitations with regard to flavor, texture, and color. A mildly heated paneer in hermetically sealed containers, with the desired flavor (like prepared fresh), color (little browning), and texture (not too hard) was developed [37]. This product was stabilized by hurdle technology and thus is stable and safe for several weeks without refrigeration. The following combinations of hurdles proved effective with this product: a_w = 0.97, heating to F_o of 0.8, pH = 5.0, or alternatively a_w = 0.96, F_o = 0.4, pH = 5.0 [37]. Fried paneer in cubes made from buffalo milk with gravy was packed either in tins or flexible retort pouches, and a set of hurdles, i.e., a_w = 0.95, F_o = 0.8, pH 5.0, and 0.1% potassium sorbate, was chosen, which had maximum lethal and inhibitory effects on microorganisms and minimum effects on textural and chemical characteristics (Table 16.7) [36]. The water activity of paneer and gravy was lowered using humectants, such as dahi, skim milk powder, salt, and glycerol. The pH was adjusted by changing the dahi:skim milk powder ratio. The resulting product had a keeping quality of 2 weeks at 45°C, 1 month at 30°C, or over 3 months at 15°C, which was limited by textural changes (hardness, cohesiveness, gumminess, springiness, and chewiness) as well as by chemical changes (browning, oxidation, lipolysis, and loss of available lysine), but not by microbial spoilage. The stabilized product was compared with fresh samples from restaurants and was found to be equally acceptable. This method of paneer preservation has a large scope for alterations in product formulations depending on regional taste preferences, without affecting the keeping quality of the product [36]. Via paneer, the hurdle technology has been introduced into food science of India, and its application to other indigenous foods is now anticipated. Further examples of the products with their hurdles and shelf life are presented in Table 16.8.

A recent example is dudh churpi. This is a popular dairy product of the Himalaya region of India (i.e., Bhutan, Sikkim, Darjeeling) made of milk from yaks or cows, and it is stable for several months without refrigeration. Most important for dudh churpi is the texture (elasticity), since people living in high altitudes chew it as an "energy tablet." The sensory quality and microbial stability of dudh churpi were optimized by Hossain [78] using combined methods (hurdle technology), that is, heating, acid coagulation, addition of sugar and sorbate, smoking, drying, and packaging in closed containers. In this detailed and diligent study, dudh churpi was scientifically explored and then a feasible optimization of the product was suggested. Thus, hurdle technology was applied to improve the quality of a traditional food of the remote Himalaya region, and at the same time the scientific basis of this study opened new avenues for food science and industrial food production in India.

16.4.5 New Technologies as Hurdles

Pulsed electric field and microfiltration as cold hurdles were used effectively instead of conventional thermal processing [79]. Milk processed with hurdles as pulsed electric field and microfiltration could produce greater microbial inactivation and overall similar shelf stability at lower processing temperatures as compared to thermal processing. This could retain better sensory properties. Although novel technologies are being used to develop hurdle products, one of the main purposes of the hurdle technologies is to use simple, easy, cheap, and low-cost methods. However, all new technologies need huge capital investments (i.e., costs), systems are complex and not readily available, and safety and design aspects are not well documented.

16.5 DESIGN OF HURDLE TECHNOLOGY FOODS

The application of hurdle technology is useful for the optimization of traditional foods as well as in the development of novel products. There are similarities to the concepts of predictive microbiology and hazard analysis critical control point (HACCP). The three concepts have related but different goals: hurdle technology is primarily used in food design, the predictive microbiology for process refinement, and the HACCP concept for process control. In product development, these three concepts should be combined. Therefore, we have suggested for the design of foods a ten-step procedure, which comprises all three concepts (Table 16.9), and this approach proved suitable when solving real product development tasks in the food industry [9, 32, 80]. However, these 10 steps should still be considered tentative until further practical experience with the application of the suggested user guide for food design has accumulated in the food industry.

In food design, different disciplines, including microbiologists and technologists, must work together. The microbiologist should determine which types and intensity of hurdles are needed for the necessary safety and stability of a particular food product, and the technologist should determine which ingredients or processes are proper for establishing these hurdles in a food (i.e., taking the legal, technological, sensory, and nutritive limitations). Because the engineering, economic, and marketing aspects have to be taken into consideration too, food design is indeed a multidisciplinary endeavor [3, 9].

Predictive microbiology [81, 82] is a promising concept that allows computer-based and quantitative predictions of microbial growth, survival, and death in foods, and thus should be an integral part of advanced food design (see Table 16.9, steps 4 and 7). However, the predictive models constructed so far handle only up to four different factors (hurdles) simultaneously. But there are numerous hurdles to be considered that are important for the stability, safety, and quality of a particular food, and need to be included in the model. It is unlikely that all or even a majority of these hurdles could be covered by predictive modeling. Thus, predictive microbiology cannot be a quantitative approach to the totality of hurdle technology.

TABLE 16.8
Examples of Hurdle Products

Food or Media	Hurdle	Shelf Life	Reference
Cane juice	Pasteurized at 80ºC for 10 min PMS (150 ppm) Citric acid (0.05%) Sterilization 80°C for 20 min Irradiation: 1.0 kGy Packed glass/PET/LDPE bottle Quality good in glass bottle Microflora: indigenous	RT: 60 days LT: 90 days	Sankhla et al. [86]
Shrimp (ready-to-eat)	a_w: 0.85 Marinated and cooked Irradiation: 2.5 kGy Storage 25°C Packaging	15 days	Kannatt et al. [27]
Assai fruit	pH 3.5 (0.5% w/w citric acid) Pasteurized at 82.5°C for 1 min Low a_w with 40% sucrose and 0.15% potassium sorbate *or* Low a_w with 25% sucrose and 0.075% potassium sorbate Storage: 25°C Light protected	150 days	Alexandre et al. [87]
Sausage	a_w: 0.95 Pasteurized 75°C or 80°C at center Storage RT	150 days	Santos et al. [88]
Pineapple or mango	Blanched 85°C for 5 min Dipped in syrup (340 ppm PMS and 413 ppm SB) 8 h Sulfur dioxide 65–85 ppm and benzoic acid 150–200 ppm Packed in polypropylene (150 gauge)	30 days (27°C) 60 days (2°C)	Vijayanand et al. [89]
Papaya	Blanched 85°C for 5 min Dipped in syrup (680 ppm PMS and 826 ppm SB) 8 h Packed in polypropylene (150 gauge) Storage 2°C or 27°C	90 days	Vijayanand et al. [89]

Notes: PMS (potassium metabisulfite); SB (sodium benzoate)

However, it does allow quite reliable predictions of the fate of microorganisms in food systems, while considering few but the most important hurdles. Because several hurdles are not taken into account, the predicted results are fortunately often on the safe side, i.e., the limits indicated for growth of pathogens in foods by the models available are in general more prudent (“fail-safe”) than the limits in real foods. Nevertheless, predictive microbiology will be an important tool for advanced food design, because it can considerably narrow the range over which challenge tests with relevant microorganisms need to be performed. Although predictive microbiology will never render challenge testing obsolete, it may greatly reduce both time and costs spent in product development [3, 9, 13].

HACCP could be a reasonable supplement to hurdle technology because after the food has been properly designed, its manufacturing process must be effectively controlled, and for this purpose, the application of HACCP might be suitable. However, in a strict sense, the HACCP concept only controls the hazards of foods and not at the same time their stability and quality [83]. Even in commercial practice, safety and quality issues will often overlap if HACCP is applied [9]. Since for hurdle technology foods the microbial safety and stability as well as the sensory quality, i.e., the total quality of the food (see Section 16.2.3), are essential, the HACCP concept might be too narrow for this purpose if it relates only to biological, chemical, and physical hazards. Therefore, the HACCP concept should be broadened in order to cover the microbial safety (food poisoning) and stability (spoilage) of foods as well as their sensory quality. If this is not acceptable, instead with HACCP the production process should be controlled by GMP, and for this purpose GMP rules or guidelines for the production of each food item must be defined [22]. For hurdle technology foods of developing countries, GMP guidelines are often more acceptable, because the application of HACCP faces practical difficulties where many small producers prevail.

TABLE 16.9
Steps for Food Design Using an Integrated Concept Comprising Hurdle Technology, Predictive Microbiology, and HACCP or GMP

Step 1	For the modified or novel food product, the desired sensor properties and the desired shelf life are tentatively defined.
Step 2	A feasible technology for the production of this food has to be outlined.
Step 3	The food is manufactured according to this technology, and the resulting product is analyzed for pH, a_w, preservatives, or other inhibitory factors. Temperatures for heating (if intended) and storage, as well as the expected shelf life are defined.
Step 4	For preliminary microbial stability testing of the food product, predictive microbiology might be employed.
Step 5	The product is challenged with food poisoning and spoilage microorganisms, using somewhat higher inocula and storage temperatures than would be "normal" for this food.
Step 6	If necessary, the hurdles in the product are modified, taking "multitarget preservation" and the sensory quality of the food (i.e., "total quality") into consideration.
Step 7	The food is again challenged with relevant microorganisms, and if necessary, the hurdles in the food are modified again. Predictive microbiology for assessing the safety of the food might be helpful at this stage.
Step 8	After the established hurdles of the modified or novel foods are exactly defined, including tolerances, the methods for monitoring the process are agreed on. Preferably, physical methods for monitoring should be used.
Step 9	The designed food should now be produced under industrial conditions, because the possibilities for a scale-up of the proposed manufacturing process must be validated.
Step 10	For the industrial process, the critical control points (CCPs) and their monitoring have to be established, and therefore the manufacturing process should be controlled by HACCP. If HACCP is not appropriate, guidelines for the application of manufacturing control by GMP must be defined.

Sources: Leistner and Hechelmann [32]; Leistner [9, 80].

16.6 CONCLUSION

The stability, safety, and quality of most preserved foods are based on the empirical application of combined methods for preservation and more recently on knowingly employed hurdle technology. The deliberate and intelligent application of hurdle technology allows a gentle, but efficient preservation of safe, stable, nutritious, and tasty foods, and is advancing worldwide. Moreover, knowledge of the physiological basis of growth, survival, and death of pathogenic and spoilage microorganisms in foods is increasing, since the homeostasis, metabolic exhaustion, and stress reactions of microorganisms in relation to the hurdle effect are now better understood. Therefore, the future achievement of multitarget preservation, the ultimate goal for gentle but effective preservation of foods, is becoming more likely.

In industrialized countries, the hurdle technology approach is at present of most interest for minimally processed, fresh-like foods, which are mildly heated or fermented, and for underpinning the microbial stability and safety of foods coming from future lines, such as healthful foods with less fat and/or salt or advanced hurdle technology foods that require less packaging. For refrigerated foods, chill temperatures are the major and sometimes the only hurdle. However, in the case of temperature abuse during distribution, this hurdle breaks down, thus spoilage or food poisoning could happen. Therefore, additional hurdles should be incorporated as safeguards into chilled foods, and this approach might be called "invisible technology."

In developing countries, the intentional application of hurdle technology for foods, which remain stable, safe, and tasty even if stored without refrigeration, has made impressive strides, especially in Latin America with the development of novel high-moisture fruit products. However, much interest in intentional hurdle technology is also emerging for meat products in China as well as for dairy products of India. There is a general trend in developing countries to gradually move away from intermediate-moisture foods, because they are often too salty or too sweet, and have a less appealing texture and appearance than high-moisture foods. However, deliberate hurdle technology should be applied to high-moisture foods without sacrificing the microbial stability and safety, especially of those foods, which are stored without refrigeration. Therefore, if hurdle technology foods become more sophisticated, they will require a thorough understanding of the principles involved as well as more backup of their production by guidelines based on GMP and, where appropriate, by application of the HACCP concept.

Hurdle technology foods are in general less robust than traditional food products, which are often overprocessed and thus have a large margin of safety. Therefore, if modified hurdle technology foods are produced the applied processes must be exactly defined and controlled. For the design of hurdle technology foods a ten-step procedure has been suggested that comprises hurdle technology, predictive microbiology, and HACCP or GMP guidelines. This procedure proved suitable for solving real product development tasks in the food industry but is open to further improvements. Hurdle technology should not lead to the addition of too many additives but should instead reduce the number of additives used even if their number might increase. It is of paramount importance that additional hurdles are introduced into a food product only after careful consideration of the essential amounts, otherwise an undesirable chemical overloading of the food might result.

Combined methods used for tissue preservation are by no means a new process, as has been pointed out by Chirife et al. [84] in their study on the mummification in Ancient Egypt. In the opinion of these authors the embalmed mummies contained >3000 years ago (at least) three hurdles, namely, reduced a_w (0.72), increased pH (10.6), and preservatives (spices, aromatic plants). However, recently, the action of combined preservative factors is being understood and their intentional and intelligent application is progressing, and further applications of hurdle technology for optimization of traditional as well as in the design of novel foods are anticipated.

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Part III

Preservation Using Chemicals and Microbes

17 Fermentation as a Food Biopreservation Technique

Nejib Guizani, Ismail M. Al Bulushi, and Ann Mothershaw

CONTENTS

17.1 INTRODUCTION

17.1.1 Fermentation Definition

Fermentation could be described as a process in which microorganisms change the sensory (flavor, odor, texture etc.) and/or functional properties (i.e., microbial health benefits) of a food to produce a desired product to the consumer. In this chapter, particular emphasis is placed on how these changes are beneficial to extend the shelf life and influence the safety of the product.

17.1.2 History

The rapidly increasing world population necessitates that the amount of food wasted needs to be kept a minimum. Food production is only one part of the process to ensure continuous,

diverse, and safe food supplies to meet consumer demand. Food must also be stored and preserved to achieve this objective. The requirement to store and preserve foods has long been recognized, even before any knowledge of microbiology. Fermentation along with cooking, smoking, and sun drying is one of the earliest ancient traditions developed by cultures all around the world to extend the possible storage time of foods. Before the initiation of preservation technology, man frequently had to choose between starvation and eating spoilt foods and then suffering the possible consequences of this. For thousands of years, raw animal and plant ingredients have been fermented. Fermented fruits were probably among the first fermented foods eaten 1, 2]. The methods for fermentations were developed by trial and error, and from the experiences of many generations.

17.1.3 Common Fermented Foods

A selection of the most common fermented foods, which has wide geographical distributions, is shown in Table 17.1. The key types of microorganisms associated with these foods are also included. Yeasts and lactic acid bacteria (LAB) are the most important groups of microorganisms. These types of microorganisms are widely used to process and preserve foods, and to produce certain types of flavors. LAB are of great industrial importance since they play an important role in the process of maturation, processing, and preserving foods. They are commonly used as starter cultures for vegetal, cereal, dairy, or meat-based products to carry out fermentations under controlled conditions. Molds, on the other hand, have limited uses in fermentation and their role is to produce desired flavor and color in certain dairy products and fermented Asian foods.

TABLE 17.1
Examples of the More Common Fermented Foods

Food	Principal Ingredient	Key Microorganisms
Wine	Grapes	Yeasts
Beer	Barley	Yeasts
Cider	Apples	Yeasts
Sake	Rice	Molds
Bread	Wheat	Yeasts
Yogurt	Milk	LAB
Cheese	Milk	LAB
Buttermilk	Milk	LAB
Kefir	Milk	LAB + yeasts
Vinegar	Grapes	Yeasts followed by *Acetobacter* + *Gluconobacter*
Tempeh	Soya beans	Molds
Soy sauce	Soya beans	Molds + LAB + yeasts
Pickled cucumbers	Cucumbers	LAB + yeasts
Sauerkraut	Cabbage	LAB
Pickled olives	Olives	LAB + yeasts
Fermented sausages	Meat	LAB + molds

Note: LAB, lactic acid bacteria.

17.2 MECHANISMS OF FOOD PRESERVATION BY FERMENTATION

In many instances, the importance of fermentation processes for food preservation has declined as new preservation techniques have been developed. Yet fermentation can be effective at extending the shelf life of foods and can often be carried out with relatively inexpensive and basic equipment. Therefore, it remains a very appropriate method for developing countries and rural communities with limited facilities. In addition, the nondependence of fermentation on the use of chemical additives to the food appeals to the "more aware" consumer market. The chemical composition of most foods is relatively stable; therefore, generally, preservation is based on eliminating microorganisms, or controlling their growth and the overall composition of the microflora. To reduce or prevent microbial spoilage of food, four basic principles can be applied:

1. Minimize the level of microbial contamination onto the food, particularly from "high-risk" sources.
2. Inhibit the growth of the contaminating microflora.
3. Kill the contaminating microorganisms.
4. Remove the contaminating microorganisms.

Fermentations use a combination of the first three principles. Fermentations should not be expected to sterilize substandard raw products, but rather should use high-quality substrates. Microorganisms can improve their own competitiveness by changing the environment so that it becomes inhibitory or even lethal to other organisms whilst stimulating their own growth, and this selection is the basis for preservation by fermentation. A number of different bactericidal and bacteriostatic factors that can be produced by LAB are shown in Table 17.2.

Fermentation can also prevent or reduce food spoilage by chemical agents. For instance, certain LAB such as

TABLE 17.2
Factors Produced by the Metabolic Activity of Microorganisms That Can Contribute to the Increased Stability and Safety of Fermented Foods

Low pH
- Organic acids, e.g., lactic acid, acetic acid, and formic acid
- Low redox potential
- Nutrient depletion (16)
- Accumulation of inhibitors, e.g., toxins, bacteriocins (183), antibiotics, lantibiotics, lactococcins, nisin, natamycin, hydrogen peroxide
- Ethanol
- Diacetyl
- Carbon dioxide

Source: Adams and Moss [30].

Lactobacillus plantarum, Lactobacillus casei subs casei, and *Pediococcus pentosaceus* were found to suppress the production of thiobarbituric acid reactive substances (TBARS), total volatile base nitrogen (TVB-N), and trimethylamine (TMA) levels [3–5]. Inhibition of the production of TBARS by LAB was explained by their capacity to scavenge oxygen (O_2), whereas their ability to suppress the increase of TVB-N and TMA was attributed to the preservative effects of LAB metabolic products and LAB antimicrobial activity [3, 6, 7].

Fermentation improves the safety of foods by reducing the risk of pathogens growing, thus limiting the chances of pathogens achieving their minimum infective dose in addition to preventing the consequential production of their metabolic products including toxins. This growth inhibition is, in part, achieved by the activity of the metabolic end-products and bacteriocins produced by the LAB. In general, bacteriocin modes of actions include inhibition of proteins synthesis and stopping synthesis of DNA and RNA in the target/pathogen bacteria [8, 9]. Different LAB have highly effective antimicrobial activities against many pathogens. For instance, *Lactococcus lactis* and *Enterococcus faecium* isolated from some fermented milk products demonstrated inhibitory action against different strains of *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Bacillus cereus* [10, 11]. In this respect, Enterocin R69 was found to have a specificity of action against *Listeria* spp., whereas Enterocin R18 had a broad spectrum of activity. *Lactococcus lactis* R9/2 and *E. faecalis* R18 induced a 2 log reduction in *L. monocytogenes* ATCC 15313 population [10]. In terms of preventing health risks from harmful microbial metabolites, certain types of LAB such as *Tetragenococcus halophilus* were found to impede the formation of histamine [7].

17.2.1 Microbial Contamination of Foods

Foods are derived from other living organisms and during their development and preparation, and these are continuously exposed to microbial contamination. The resultant contaminating microflora can have different effects on the food. These include negative effects such as spoilage, where the food becomes unfit for human consumption or health risks when infectious or toxigenic microorganisms are present. Negligible effects on the food occur when the microflora does not cause disease or any detectable changes in the food. However, benefits can also be reaped from the action of the microorganisms when their activity brings about improvements in the appeal (i.e., desired or preferred sensory attributes) of the food. In developed countries, the improved appeal is the major reason for microbial fermentation of foods continuing today.

The nutrient content and intrinsic properties of many raw foods make them ideal environments for microbial replication. The rate at which the microorganisms grow depends not only on the intrinsic properties of the food (pH, redox potential, water activity, etc.) but also on the conditions under which it is being stored, the extrinsic factors, e.g., temperature. Therefore, many raw foods need to be consumed soon after production to retain their nutritional value. The delays in preservation measures lead to degradation of the nutrients and utilization by the contaminating microflora.

A major consideration needs to be given to ideal conditions, since microorganisms can grow very rapidly, being able to double in number in a short period. It must also be noted that there is a variation in the optimum environmental conditions for different types and species of microorganisms, e.g., microorganisms can be categorized into broad groups such as aerobes and anaerobes depending on their tolerance and use of oxygen, and psychrophiles, mesophiles, and thermophiles based on temperature growth requirements. In addition, the biochemical activity of different microorganisms varies and may change in response to fluctuations in environmental factors, leading to a range of metabolic end products (Table 17.3). By manipulating the environmental conditions, it is possible to select specific kinds of microorganisms that impart a particular taste, odor, texture, or appearance to the food. This is one of the important bases of fermentation.

17.2.2 Benefits of Fermented Foods

Microbial applications in food industry are of major interest. The acceptability of a food to the consumer is based mainly on its sensory properties when food safety is achieved. The sensory properties of fermented foods are brought by the biochemical activity of microorganisms. Fermented foods were developed simultaneously by many cultures for two main reasons: (i) to preserve harvested or slaughtered products, which were abundant at certain times and scarce at others, and (ii) to improve the sensory properties of an abundant or unappealing product [1, 12].

The range of benefits that can be obtained from fermentation of foods can be extended to include preservation through formation of inhibitory metabolites such as organic acids (lactic acid, acetic acid, formic acid, and propionic acid), ethanol, bacteriocins. This activity is frequently enhanced by combining with (i) decreased water activity (by drying or salting) [13, 14], (ii) improving food safety through inhibition of pathogens [15] or removal of toxic compounds [16], (iii) improving the nutritional value [17, 18], (iv) improving organoleptic quality of food [1, 12, 19, 20], and (v) consumer acceptance due to its natural process without the addition of synthetic chemicals.

TABLE 17.3
Examples of Microbial Metabolic End Products Used in Fermented Foods

Metabolic End Product	Example of Uses
Carbon dioxide	Leavening bread
Ethanol	Alcoholic beverages
Acids	Vinegar
Acetic	Fermented vegetables
Lactic	
Flavor compounds	Dairy products
Acetaldehyde	Yoghurt

Fermentation has low energy demands and can often be carried out without sophisticated technology and designated plants. The simple techniques mean that the procedures can often be carried out in the home [21]. Fermented foods and drinks retain an important role in the human diet. A number of studies have shown that consumers regard fermented food products as healthy and natural, and increasing consumer demand makes profitability [22]. Consumer interest in probiotic foods, which include live LAB, has increased due to their health benefits. Potential health benefits from the activity of probiotic foods include hydrolysis of lactose, reduced serum cholesterol levels, reduced colon cancer, improved immune response and reduced allergic diseases [23].

In fermentation of food wastes, a number of useful chemicals have been produced. For instance, volatile fatty acids yield of 30.22 g/L and improve acetic acid production to 25.88 g/L were produced by yeasts and acetic acid bacteria fermentation of food wastes (24). Using *Rhodobacter sphaeroides* as a fermenter, average yields of hydrogen was approximately 199 ml hydrogen/g cassava and 220 ml hydrogen/g food waste were produced by a two-step process of dark fermentation and photofermentation, respectively [25].

17.3 MICROORGANISMS USED IN FOOD FERMENTATIONS

Various groups of microorganisms are frequently used in fermented foods and the principal groups are shown in Table 17.4.

17.3.1 Lactic Acid Bacteria

Lactic acid bacteria perform an essential role in the preservation and production of wholesome foods. Examples of lactic acid fermentations include (i) fermented vegetables, such as sauerkraut, pickled cucumbers, radishes, carrots, and olives; (ii) fermented milks, such as yogurt, kefir, and cheeses; (iii) fermented/leavened breads, such as sourdough breads; and (iv) fermented sausages (Table 17.1). LAB have been grouped together as they possess a range of common properties (Table 17.5) and all produce lactic acid, which can kill or inhibit many other microorganisms [26]. The primary use of lactic acid in the food industry is as a preservative, an acidulant, or a dough conditioner and raiser. The principal genera of lactic acid bacteria are shown in Table 17.6. In general, excluding some streptococci, they are harmless to humans. This makes lactic acid bacteria ideal agents for food preservation. LAB are subdivided based on their products from glucose fermentation. Homofermenters produce lactic acid as the major or sole product from glucose, whereas heterofermenters produce equimolar amounts of lactate, carbon dioxide, and ethanol. Heterofermenters have an important role in producing aroma components, such as acetaldehydes and diacetyl. LAB have a range of methods for outcompeting other microorganisms (Table 17.2). Their most effective mechanism is to grow readily in most foods, producing acid, which rapidly lowers the pH to a point where other competing organisms can no longer grow [12]. Lactobacilli also have the ability to produce hydrogen peroxide [27], which is inhibitory to spoilage organisms [12], whilst lactobacilli are relatively resistant to hydrogen peroxide [28]. The role of hydrogen peroxide as a preservative is likely to be minor, especially when compared to acid production. Carbon dioxide produced by heterofermenters also has a preservative effect resulting partially from its contribution to anaerobiosis [12].

Consumers are taking a greater interest in the quality of foods and are creating a demand for chemical-free, "natural health" foods. This has stimulated extensive research into the applications of lactic acid bacteria for both the control of pathogenic and spoilage microorganisms and also for health promotion. A range of potential health benefits has been associated with the consumption of LAB. Some benefits due to a consequence of their growth and activity during food fermentations and some from the resultant colonization of the gastrointestinal tract (Table 17.7). Many of these health claims are still controversial [23]) and are the subject of research to identify and substantiate specific roles [16, 23, 29].

TABLE 17.4
Principal Groups of Microorganisms Used for Food Fermentation

Microbial Group	Product
Lactic acid bacteria (LAB)	Lactic acid
Acetic acid bacteria	Acetic acid
Yeasts	Alcohol and carbon dioxide
Molds	Enzymes

TABLE 17.5
Characteristics Common to Lactic Acid Bacteria (LAB)

Gram-positive
Catalase negative
Oxidase negative
Non-spore forming
Fermentative anaerobes that are aerotolerant
Produce most of their cellular energy from the fermentation of sugars
Produce lactic acid from hexoses

17.3.2 Acetic Acid Bacteria

A second group of bacteria with importance in food fermentations is the acetic acid producers. Acetic acid is one of the oldest chemicals known; it is named after the Latin word for "vinegar," *acetum.* The acetic acid bacteria are acid-tolerant; grow well at pH levels below pH 5.0; are gram-negative, motile rods; and are obligate aerobes. They derive energy from the oxidation of ethanol to acetic acid following the reaction

TABLE 17.6
Genera of Lactic Acid Bacteria Commonly Used in Food Fermentations

Genus	Cell Shape and Grouping	Homofermenter	Heterofermenter
Lactobacillus	Rods, single or chains	+	+
Lactococcus	Oval cocci, pairs or chains	+	–
Leuconostoc	Oval cocci, pairs or chains	–	+
Pediococcus	Cocci, pairs and tetrads	+	–
Streptococcus	Cocci, pairs and chains	+	–
Weissella	Coccoid/short rods; single, pairs, or short chains	–	+
Enterococcus	Cocci; single, pairs, or short chains	+	–

Sources: Modified from Axellson [26] and Adams and Moss [30].

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

They are found in nature where ethanol is being produced from the fermentation of carbohydrates by yeasts, such as in plant nectars and damaged fruits. Other good sources are alcoholic beverages like fresh cider and unpasteurized beer. In liquids, these grow as a surface film due to their demand for oxygen.

Acetic acid bacteria consist of two genera: *Acetobacter* and *Gluconobacter*. *Acetobacter* can eventually oxidize acetic acid to carbon dioxide and water using Kreb cycle enzymes, referred to as overoxidation. This is not the case with *Gluconobacter*. The most desirable action of acetic acid bacteria is in the production of vinegar. The same reaction can also occur in wines, when oxygen is available, and here the oxidation of alcohol to acetic acid is an undesirable change, giving the wine a vinegary off-taste.

17.3.3 Yeasts

Yeasts are widely distributed in natural habitats that are nutritionally rich and high in carbohydrates, such as fruits and plant nectars [30]. Yeasts are rarely toxic or pathogenic, and are generally acceptable to consumers [31]. After extensive study, yeasts have been classified into about 500 species [32]. However, only a small number are regularly used to make alcoholic beverages [30]. *Saccharomyces cerevisiae* is the most frequently used and many variants are available. *S. cerevisiae* ferments glucose but does not ferment lactose or starch directly. Yeasts are used to produce ethanol, carbon dioxide, flavor, and aroma. The reaction can be represented by the following equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

$$\text{yeast} + \text{glucose} \rightarrow \text{ethyl alcohol and carbon dioxide}$$

Other metabolic products include minor amounts of ethyl acetate, fusel alcohols (pentanol, isopentanol, and isobutanol), sulfur compounds, and leakage of amino acids and nucleotides can all contribute to the sensory changes induced by yeasts [31].

TABLE 17.7
Potential Health Benefits from Lactic Acid Bacteria

Benefits
From Foods
Improved nutritional value, e.g., production of vitamins or essential amino acids
Reduced toxicity, e.g., by degradation of noxious compounds
Increased digestibility and assimilability of nutrients (23)
From Colonization
Control of intestinal infections
Improved digestion of lactose
Inhibition of tumor growth
Lowering of serum cholesterol levels
Immune stimulation (184)

Source: Drouault and Corthier [185].

17.3.4 Molds

The majority of fungal species has filamentous hyphae and it is referred to as molds. They are grouped into four main classes based upon the physiology and production methods of their spores. Molds are aerobic and have the greatest array of enzymes. Some molds are used in the food industry to produce specific enzymes, such as amylases for use in bread making. They are relatively tolerant to extreme environments and are able to colonize and grow on most foods. Molds are important to the food industry, both as spoilers and preservers of foods and in particular in fermentations for flavor development. Certain molds produce antibiotics [33, 34], whereas mycotoxin production by others is an emerging cause of concern in the food industry.

The *Aspergillus* species are often responsible for undesirable changes in foods, although some species such as *Aspergillus oryzae* are used in fermentations of soybeans to make miso and soy sauce. *Mucor* and *Rhizopus* are also used in some traditional food fermentations. *Rhizopus oligosporus* is considered essential in the production of tempeh from soybeans. Molds from the genus *Penicillium* are associated

with the ripening and distinctive flavor of cheeses. For example, during ripening of Roqueforte and blue cheeses, *Penicillium roqueforti* is grown in air veins throughout the curd and the distinctive flavors develop as the milk lipids are broken down into methyl ethyl ketone and proteins are structurally altered.

17.3.5 Starter Cultures

Fermented foods may be produced by the action of fermentative microorganisms naturally found on the raw materials or in the production environment. However, "starter cultures" are frequently used to improve reliability. Starter cultures may be pure or mixed cultures. Mixed starter cultures can reduce the risks of bacteriophage infection [35] and improve the quality of the foods when the organisms are mutually beneficial. Food fermentations frequently involve a complex succession of microorganisms induced by dynamic environmental conditions. Fermentative microorganisms must be safe to eat even in high numbers and must produce substantial amounts of the desired end product(s).

To ensure food safety, starter cultures must be carefully selected to avoid incorporation of LAB metabolic products that could pose health risks following consumption of the food products. For example, organisms that can produce biogenic amines by possessing amino acid decarboxylase activity should be avoided [36]. Other criteria that could be considered in selection of the starter culture may include acid and bile tolerance; intestinal epithelial adhesion properties (for efficacy as probiotics); production of antimicrobial substances that can kill or inhibit pathogens and spoilage microorganisms; antibiotic resistance patterns; and immunogenicity [37].

For practical reasons, the organisms should be easy to handle and grow well, enabling them to outcompete undesirable microorganisms. The organism also needs to be genetically stable with consistent performance both during and between food batches. In many traditional fermentations the natural microflora were used for the fermentation. Even so, some form of inoculation was frequently performed using simple techniques like the use of one batch of food to inoculate the next batch or the repeated use of the same container [38]. Natural fermentations have a degree of unpredictability, which may be unsatisfactory when a process is industrialized. Starter cultures are increasingly used to improve not only the reliability, but also the reproducibility and the rate at which the fermentation is initiated. Failed, poor quality, or unsafe products lead to loss of customers and revenue, therefore their incidence must be minimized.

The composition of starter cultures is based on the knowledge of food-grade microbial genetics [39, 40], metabolism, and physiology as well as their interactions with foods [40]. Starter cultures are now developed mainly by design rather than by screening [41, 42]. The overall objective is to exploit the properties of the starter cultures to ensure reproducible standards of safety and quality [43].

17.4 CLASSIFICATION OF FERMENTED PRODUCTS

Fermented foods are classified in a number of different ways. They may be grouped based on the microorganisms, the biochemistry, or on the product type [44]. Campbell-Platt identified seven groups for classification, namely, (i) beverages, (ii) cereal products, (iii) dairy products, (iv) fish products, (v) fruit and vegetable products, (vi) legumes, and (vii) meat products [45]. Steinkraus classified fermentations according to the methods of fermentation, e.g., alcoholic (wines and beers) and alkaline (Nigerian dawadawa) [46]. In this chapter, the fermentations are grouped in terms of the biochemical products used to transform the food, i.e., production of lactic acid, acetic acid, ethanol, and/or carbon dioxide.

17.5 FERMENTED PRODUCTS

17.5.1 Alcoholic Beverages

Throughout history, alcoholic beverages have had a place in most cultures. They require the alcoholic fermentation of fruits or other high-sugar materials by yeasts. The alcohol content of the beverage acts as a preservative and many of these products have long shelf lives. Over the years, brewing yeasts have evolved by selection and mutations, and have been developed by genetic engineering. Major advances have been made in improving the characteristics of the fermentation strains driven by the high revenue associated with the alcoholic beverage industry.

17.5.1.1 Beer

Beer is produced by the fermentation of partially germinated cereal grains, referred to as malt, by yeasts. Beers have a final ethanol content of about 3–8%. A huge variety of beers exists, and these include ales, lagers, and stouts. Both lagers and ales can be either light or dark in appearance. Ale is produced using *Saccharomyces cerevisiae,* a top fermenter yeast, whereas lagers are produced using pure cultures of *Saccharomyces carlsbergensi*, a bottom fermenter yeast. Ales are produced using warm fermentation temperatures (12–18°C), and lager fermentation temperature is generally cold (8–12°C) [30]. Most beer produced is of the lager variety.

Several steps are needed to make beer. First, the barley is soaked in water for 5 to 7 days in order to make malt [47, 48]. During this step, the grains partially germinate and produce enzymes, mainly amylase and protease, that are essential to the brewing process. Amylases degrade starch to glucose, a sugar needed for the yeast fermentation, and proteases solubilize compounds in the grain and hops, which is important for the quality of beer. Following germination, heat is applied to stop further sprouting and to dry the grain. To develop color and aroma, the malt is roasted for 4–5 hours at a temperature of 80–105°C. Maillard reactions are responsible for the color and aroma formation during kilning. The dried and crushed malt is suspended in water and mixed with boiled malt adjuncts, such as ground rice and corn. Amylase is

generally added at this stage to ensure complete hydrolysis of starch. The mash is then incubated at 65–70°C for a short time to allow the amylase to degrade the starch to glucose. The temperature is subsequently raised to 75°C to inactivate the enzymes, and the medium is allowed to settle. Insoluble matter sinks to the bottom and serves as a filter as the liquid, called wort, is taken from the container. Hops or hop extracts are then added to the wort. Hops are an indispensable ingredient, as they act as a clarifier causing protein to precipitate; they give a specific aroma and bitter taste. Hops also possess antibiotic properties and together with ethanol and carbon dioxide contribute to the stability of beer [49–51]. In addition, the protein content of hops enhances the foam-building ability of beer. The mixture is boiled for 1.5–2.5 h to obtain the correct delicate hop flavor [52]. The wort/hops mixture is then boiled in order to concentrate the wort, kill many spoilage microorganisms, inactivate enzymes in the mash, and solubilize important compounds in the hops and mash. The wort is then separated, cooled, and fermented.

Fermentation is initiated by adding the appropriate yeast to the wort. Ale fermentation is completed when the pH is lowered to around 3.8, generally in 7–12 days; lager has pH values of 4.1–4.2 and the process is completed in 5–7 days [53]. During fermentation, the glucose in wort is converted to ethanol and carbon dioxide. The fermented wort is then aged at 0°C for a period of weeks or months. During this period, the yeast settles to the bottom of the vessel, bitter flavors are mellowed, and other compounds are formed that enhance flavor. The beer is then filtered or centrifuged to remove yeast cells before packing and pasteurization. The beer is finished by the addition of carbon dioxide to a final content of 0.45% to 0.52%. Finally, pasteurization of the beer at 60°C or higher, may be carried out to destroy spoilage microorganisms [54].

There are a number of factors that protect beer from the growth of contaminating microorganisms. These include low pH, redox potential and levels of readily available carbon sources, the isohumulones of hops that inhibit gram-positive bacteria, and the alcohol produced by the yeast [55]. The spoilage of beer is caused mainly by acetic acid bacteria, lactic acid bacteria, and wild yeasts. The industrial spoilage of beers is commonly referred to as beer infection [54]).

17.5.1.2 Wine

Wines can be produced from any fruit juice with sufficient levels of fermentable sugars. In most cases, wine is a beverage obtained by full or partial alcoholic fermentation of fresh, crushed grapes or grape juice (must), with an aging process. Wine-type grapes from cultivars of *Vitis vinifera* vines are the most used to produce wines [49]. Winemaking involves a series of steps. First, grape clusters are cleaned of rotten and dried berries and then separated from the stems. The grapes are subsequently crushed and pressed to release juice, the must. The remaining grape skins and seeds, called pomace, are then removed after a second press. In red winemaking, the must is fermented together with the skin in order to extract the red pigments from the skin, which are released only during fermentation. The extraction of the red pigments is sometimes facilitated by raising the temperature to 50°C prior to fermentation of the mash, or to 30°C after the main fermentation, followed by a short additional fermentation.

The fresh sweet must is treated with sulfur dioxide to suppress the growth of undesirable microorganisms and to prevent enzymatic browning and oxidation, thus stabilizing the wine color. The must is then inoculated with *Saccharomyces cerevisiae* var. *ellipsoides* or *pastorianus*, and allowed to ferment for 3–5 days at temperatures between 21°C and 32°C. During this period, ethanol production may reach 14% to 18%. Fermentation of red wine is longer than that of white wine, until the correct amount of color is extracted from the skin. The wine is racked in order to get rid of the sediments. The wine is drawn-off or decanted into barrels, vats, or tanks for aging, the length of which could vary between 3 and 9 months. During this stage, the wine clears and develops flavors. The wine is then removed from vats and poured into bottles in which aging continues [56]. Following the alcoholic fermentation, a malolactic fermentation can be initiated to reduce the acidity and mellow the wine. During the malolactic fermentation, malic acid is degraded to lactic acid by many lactic acid bacteria, mainly of the genera *Lactobacillus*, *Leuconostoc* (*L. oenos*), and *Pediococcus* (*P. cerevisiae)* [55]. Lactic acid is not as acidic as malic acid, hence the acidity of the wine is reduced. Wine can be subjected to some microbial and chemical defects. Microbial spoilage can be caused by the complex natural microbial flora of grapes and wine, which comprises molds [57], lactic acid bacteria [58], and acetic acid bacteria [59, 60].

The presence of acetic acid bacteria is less desirable than lactic acid bacteria and yeasts. Wines are subject to spoilage by acetic acid bacteria at many stages during the winemaking process. Spoilage by acetic acid bacteria gives wine a characteristic volatility; a vinegar-like sourness on the palate; and a range of acetic, nutty, sherry-like, solvent, or bruised apple aromas and often a reduction in fruity characters [61]. Such wines have low commercial value but can in some cases be improved by blending or treatment by a reverse-osmosis process to lower the acetic acid content [62]. Chemical defects lead mainly to the browning of wine as a result of oxidative reactions of phenolic compounds, which in red wines, may result in complete flocculation of the color pigments. [63])

17.5.2 Distilled Spirits

The aforementioned fermentations can only produce a maximum alcohol content of about 17%. Concentrations in excess of this inhibit the metabolism of the yeasts. To obtain higher alcohol concentrations, the fermented product must be subsequently distilled. Whiskeys, gin, vodka, rum, and liqueurs are examples of distilled spirits. Although the process for producing most products of these types is quite similar to that for beers, the content of alcohol in the final products is considerably higher.

17.5.3 Lactic Acid Products

17.5.3.1 Dairy Products

17.5.3.1.1 Yogurt

Yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Several other microbial species might be added to yogurt such as *Lactobacillus. acidophilus*, *Bifidobacterium lactis*, *L. casei* [64], *L. reuteri*, and *L. rhamnosus* [65]. These microorganisms make yogurt rich in probiotics, which is one of its beneficial characteristics, and the presence of probiotics does not negatively affect yogurt flavor or consumer acceptance [66].

The main quality characteristics of yogurt include texture, taste, aroma, and flavor [67–69]. Typically, yogurt is a smooth, viscous gel with a characteristic taste of sharp acid and a green apple flavor [70]. Yogurt can be made available in different forms such as drinking yogurt, lactose-hydrolyzed yogurt, strained yogurt, frozen yogurt (with categories soft, hard, or mousse), dried yogurt, bio-yogurt (yogurt made with different live cultures other than the two most widely used), vegetable oil yogurt, soy yogurt, and chemically acidified yogurt [71].

Yogurt is prepared using either whole or skim milk, where the nonfat milk solids are increased to 12–15% by concentrating the milk or adding powdered skim milk or condensed milk. The concentrated milk is pasteurized to 82–93°C for 30–60 min and cooled to the starter incubation temperature of 40–45°C. Yogurt starter is then added at a level of around 2% by volume and incubated for 3–5 h, or until the titratable acidity of the final product reaches 0.85–0.90% or a pH of 4.4–4.6 [54]. The yogurt is then cooled to 5°C to inhibit further acid production. The symbiotic growth of the two organisms of the yogurt starter culture has been reviewed by many authors [72–74]. The symbiotic growth of the two organisms is better observed when they exist in a 1:1 ratio and results in lactic acid production and acetaldehyde at a rate greater than that produced by either when growing alone [72]. Streptococci produce lactic acid, formic acid. and carbon dioxide. Formic acid stimulates the growth of lactobacilli. The lactobacilli liberate some amino acids needed for the growth of the streptococci, and produce acetaldehyde and more lactic acid to bring the pH to 4.4–4.6. Most flavor compounds in yogurt are produced from lipolysis of milk fat and microbiological transformations of lactose and citrate. More than 100 volatiles, including carbonyl compounds, alcohols, acids, esters, hydrocarbons, aromatic compounds, sulfur-containing compounds, and heterocyclic compounds, are found in yogurt at low to trace concentrations. Besides lactic acid, most of the typical aroma and flavor of yogurt are mainly due to acetaldehyde and to a lesser degree to diacetyl, acetoin, acetone, and 2-butanone, which are produced in lower concentrations [75, 76]. Yogurt flavor continuously changes during manufacture and storage. Flavor changes may vary depending on the cultures, mix formulation, and incubation and storage conditions [77]. *Lactobacillus acidophilus* may be added with yogurt culture to reduce excessive aldehyde in addition to its health benefits. The type of yogurt starter used can change the physical characteristics of the final yogurt product. For example, ropy cultures, used to enhance the viscosity of "stirred" types of yogurt, comprise *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus* strains [78]. "Non-ropy" starters are used for the manufacture of "set" types of yogurt. Other ways to increase the viscosity of yogurt and subsequently to decrease the syneresis of the whey include the addition of stabilizers, increasing nonfat milk solids, and extending the time and/or increasing the temperature of pasteurization.

The effect of the addition of fruits to the yogurt formula on lactic acid bacteria was found to depend on the type of fruits and lactic acid bacterial strains. Adding mixed berry, strawberry, mango, and passion fruit in yogurt formula and using starter culture consisting of *Lactobacillus acidophilus* LAFTIs L10 and *Bifidobacterium animalis* ssp. *lactis* LAFTIs B94, Kailasapathy et al. [79] found that the addition of either 5 or 10 g/100 g fruit preparations had no significant effect on the viability of the two probiotic strains except on *L. acidophilus* LAFTI L10 yogurt with 10 g/100 g passion fruit or mixed berry.

17.5.3.1.2 Cheese

Cheese is a concentrated milk product obtained after coagulation and whey separation of milk, cream or partially skimmed milk, buttermilk, or a mixture of these products. Cheese may be consumed fresh or after ripening. Cheese is commonly made from cow, ewe, goat, or buffalo milk. The majority of cheeses are made from pasteurized milk. The use of subpasteurization heat treatment of milk, or thermization, is also practiced in order to limit heat-induced changes in milk without compromising microbiological safety. There are over 400 varieties of cheeses representing fewer than 20 distinct types, and these are grouped or classified according to texture or moisture content, whether ripened or unripened, and if ripened, whether by bacteria or molds [54]. Table 17.8 shows the classification of cheeses according to moisture on fat-free basis, with subdivisions depending on the role of microorganisms in cheese ripening. The majority of cheeses, with the exception of whey cheeses, are made using variations of the same basic process, as illustrated in Figure 17.1. Slight variations of these processes and the use of different types of milks generate the huge range of cheeses available today.

In general, the process of manufacture starts with the preparation of milk. Milk receives generally a treatment equivalent to pasteurization at the start of the processing. The milk is then cooled to the fermentation temperature, which depends on the type of cheese to be manufactured: 29–31°C for cheddar, Stilton, Gouda, Camembert, and Leicester; higher temperatures are employed in the manufacture of high scalded cheeses such as Emmental, Gruyere, and Italian cheeses. Milk is inoculated with an appropriate lactic starter. The starter culture produces lactic acid, which, with added rennin, gives rise to curd formation. In addition, lactic acid is also responsible for the fresh acidic flavor of unripened cheeses and plays a major role in the suppression of pathogenic and some spoilage microorganisms, and in the production of volatile flavor

TABLE 17.8
Cheese Varieties and Their Classification

MFFB*	
> 67%	Soft Cheeses
	Unripened
	Cottage, Quark, Cream, Mozzarella
	Salt-cured or pickled
	Feta, Domiati
61–69%	Semisoft Cheeses
	Ripened by bacteria and surface microorganisms
	Limburger, Brick, Port du Salut
	Ripened by surface molds
	Camembert, Brie
54–63%	Semihard Cheeses
	Ripened principally by internal mold growth
	Roquefort, Stilton, Gorgonzola
	Ripened internally by bacteria
	Edam, Gouda
49–56%	Hard Cheeses
	Ripened internally by bacteria
	Without eyes: Cheddar, Caciocavallo
	With eyes: Emmental, Gruyère
	Ripened internally with molds
	Cheshire, Blue
<41%	Very Hard
	Ripened by bacteria
	Parmesan, Romano, Grana

* Percentage of moisture on fat-free basis.

compounds, and the synthesis of lipolytic and proteolytic enzymes involved in the ripening process of cheese. The starter organisms most used for cheese production are mesophilic starters, strains of *Lactococcus lactis* and its subspecies. Thermophilic starters such as *Lactobacillus helveticus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* are used in the production of cheeses where a higher incubation temperature is employed. Propionic bacteria, molds, such as *Penicillium camemberti*, *P. candidum*, and *P. roqueforti*; red- or yellow-smearing cultures, such as *Bacterium linens* are also added, depending on the type of cheese to be manufactured. The time of renneting and the amount added differ with cheese type. After coagulation of the milk, the curd is cut into small cubes for whey expulsion. The curd is further shrunk by heating it and then pressed to expel more whey, followed by salting. Finally, the cheese is ripened under conditions appropriate to the cheese in question.

Cheese ripening involves a complex series of chemical and biochemical reactions. Proteolysis and lipolysis are two primary processes in cheese ripening with a variety of chemical, physical, and microbiological changes occurring under controlled environmental conditions [80, 81]. These reactions are of importance to the flavor and texture development in cheeses [82–84]. Flavor compounds include peptides and amino acids, free fatty acids, methyl ketones, alkanes,

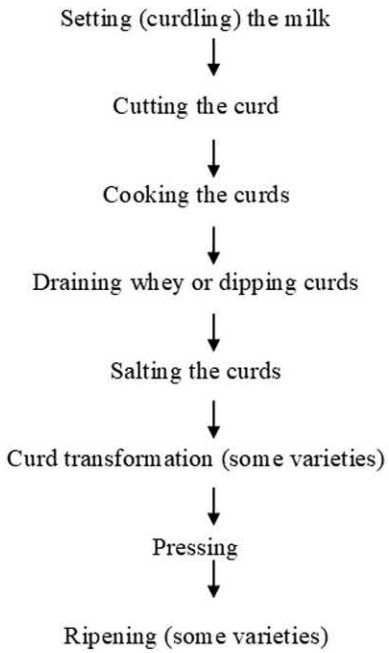

FIGURE 17.1 Basic steps in cheese making.

lactones, and aliphatic and aromatic esters. Figures 17.2 and 17.3 briefly summarize, respectively, the proteolytic and lipolytic products obtained during ripening.

Although most ripened cheeses are the products of metabolic activities of the lactic acid bacteria, several known cheeses owe their particular character to other related organisms. In the case of Swiss cheese, *Propionibacterium shermanii* is added to the lactic bacteria organisms *L. bulgaricus* and *S. thermophilus*. Propionibacteria contribute to the typical flavor and texture of Swiss-type cheeses [85]. The lipolytic and proteolytic activities of molds play an important role in the maturation of some cheeses. In blue cheese, such as Roquefort and Stilton, *Penicillium roqueforti* grows throughout the cheese and imparts the blue-veined appearance characteristic of this type of cheese. *Penicillium camemberti* is associated with surface-ripened soft cheeses such as Camembert and Brie.

17.5.3.2 Fermented Vegetables

A large number of vegetables are preserved by lactic acid fermentation around the world. The most important commercially fermented vegetables in the West are cabbage (sauerkraut), cucumbers, and olives. Others include carrots, cauliflower, celery, okra, onions, and peppers. Typically, these fermentations do not involve the use of starter cultures and rely on their natural flora. Brine solutions are prepared in the fermentation of sauerkraut, pickles, and olives. The concentration of salt in the brine ranges from 2.25% for sauerkraut to 10% for olives. The fermentation yields lactic acid as the major product. The salt extracts liquid from the vegetable, which serves as a substrate for growth of lactic acid bacteria. Growth of undesirable spoilage microorganisms is restricted by the salt. Aerobic conditions should be maintained during fermentation to allow naturally occurring microorganisms

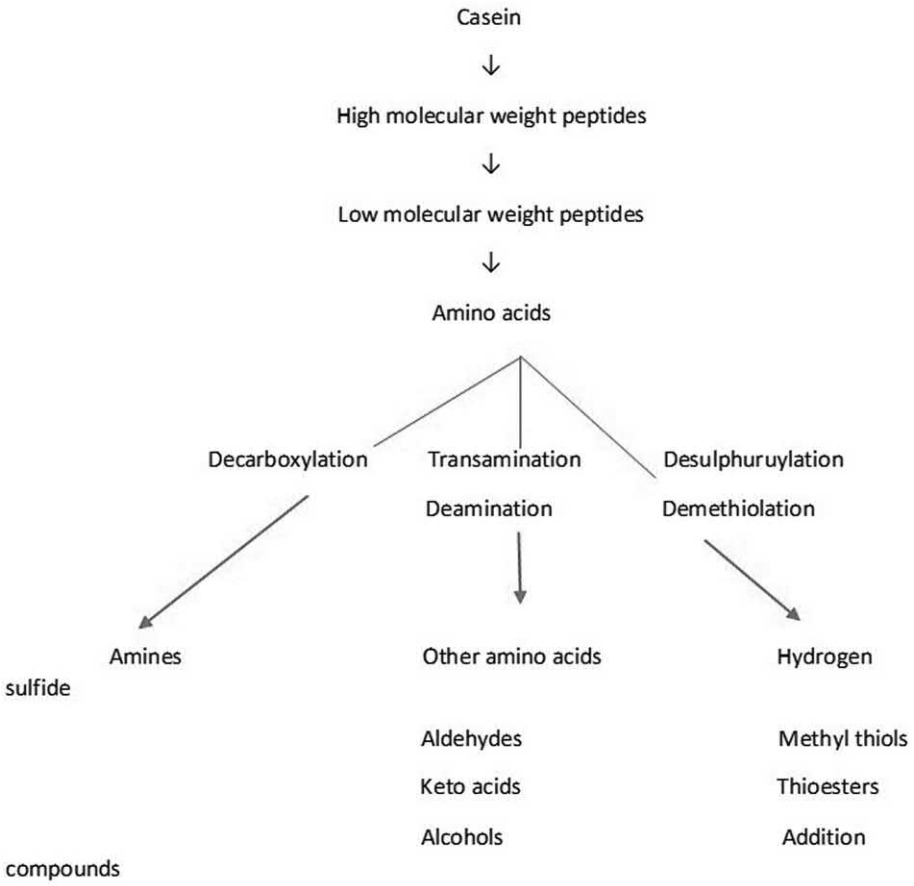

FIGURE 17. 2 Protein degradation during ripening of cheese. (Adapted from various sources.)

to grow and produce enough lactic acid, and to prevent growth of spoilage microorganisms. Olives receive a special treatment before brining, and green olives are treated with a 1.25% to 2% lye solution (sodium hydroxide), usually at 21°C to 25°C for 4 to 7 hours. This treatment is necessary to remove some of the oleuropein, a bitter compound in the olives. In some countries, the fermentation of cucumbers is controlled by the addition of acetic acid to prevent growth of spoilage microorganisms, buffered with sodium acetate or sodium hydroxide, and inoculated with *L. plantarum* alone or in association with *P. cerevisiae*. The controlled fermentation

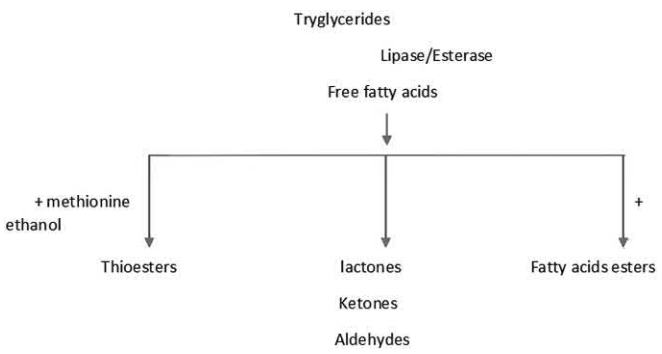

FIGURE 17.3 Lipid degradation during ripening of cheese. (Adapted from various sources.)

reduces economic losses and leads to a uniform product over a shorter period. Many researches have shown a sequential involvement for different species of lactic acid bacteria [1, 86–88]. For sauerkraut production *Leuconostoc mesenteroides* grows first, producing lactic acid, acetic acid, and CO_2; followed by *Lactobacillus brevis*; and finally *Lactobacillus plantarum* grows, producing more acid and lowering the pH to below 4.0, allowing the cabbage to be preserved for long periods of time under anaerobic conditions. The lactic acid bacteria chiefly responsible for production of high-salt pickles are initially *Pediococcus cerevisiae* followed by the more acid-tolerant *Lactobacillus plantarum* and *Lactobacillus brevis. Leuconostoc mesenteroides* makes little contribution in high-salt pickles but is active in low-salt pickles [89]. The microbiology of olive lactic acid fermentation is complex with a number of microbial strains being involved. Vaughn et al. [90] have divided the normal olive fermentation into three stages. The initial stage is the most important from the standpoint of potential spoilage if the brines are not acidified. Acidification eliminates the original contaminating population of dangerous gram-negative and gram-positive spoilage bacteria and, at the same time provides an optimum pH for activity of the lactic acid bacteria [91]. Currently, in olive fermentations starter cultures are not used, although a proportion of brine from a previous fermentation may be used to supplement the new brine. The natural flora of green olives,

consisting of a variety of bacteria, yeasts, and molds carries out the fermentation with the lactic acid bacteria becoming prominent during the intermediate stage. *Leuconostoc mesenteroides* and *Pediococcus cerevisiae* are the first lactics to predominate, followed by lactobacilli, mainly *Lactobacillus plantarum* and *Lactobacillus brevis* [92].

To enhance control of the fermentation process and to improve the quality of the final product, the use of starter cultures has been investigated. A possible role for yeasts as starters has recently been proposed for production of table olives [93, 94]. A new protocol for the production of black table olives belonging to two Italian (Cellina di Nardò and Leccino) and two Greek (Kalamàta and Conservolea) cultivars has been developed: for each table olive cultivar, starter-driven fermentations were performed inoculating, firstly, one selected autochthonous yeast starter and, subsequently, one selected autochthonous LAB starter [95].

A range of potential health benefits has been associated with the consumption of lactic acid bacteria.Fermented vegetables can be used as a potential source of probiotics, as they harbor several lactic acid bacteria such as *Lactobacillus plantarum*, *L. pentosus*, *L. brevis*, *L. acidophilus*, *L. fermentum*, *Leuconostoc fallax*, and *L. mesenteroides* [96, 97].

The consumer demand for nondairy beverages with high functional value is increasing. It is driven in part by the ongoing trend of vegetarianism and the increasing prevalence of lactose intolerance. The use of vegetable matrices as potential nondairy vehicles for delivering probiotic strains has been assessed [98, 99]. Tomato, carrot, cabbage, artichokes, and red beet juices were proven to be particularly suitable for probiotic fermentation, allowing a rapid growth of the strains and viable cell populations above ca. 8 log cfu/mL [99]. Nutrients essential for microbial growth and survival are also added to vegetable juices. *L. plantarum* and *L. acidophilus* strains showed optimal growth and acidification in beetroot juice when yeast extract was added. The careful selection of the vegetable matrix, probiotic strains and the addition of other ingredients are all biotechnology options to optimize the manufacture of probiotic, non-dairy beverages [100].

17.5.3.3 Fermented Animal Products

The primary reason for developing methods to ferment meats and fish was to extend the shelf life of these highly prized, perishable foods. Gram-positive micrococci have an important role in these fermentations [101]. Several products became popular including fermented sausages, fish sauces, and fish pastes. Many of the traditional fermentation methods are still used, although the primary reason for their use is no longer preservation but because the products are popular for their enhanced flavors.

17.5.3.3.1 Fermented Sausages

A variety of procedures for producing stable, fermented meat sausages have developed around the world. In general, preservation of the meat is achieved by adding salts and the generation of lactic acid by bacteria, which leads to a rapid fall in the pH. Micrococci, staphylococci, and yeasts are responsible for the development of color, taste, and flavor during the fermentation. In addition to fermentation, sausage processing may also include curing, smoking, drying, and aging to both improve the flavor and shelf life. In addition to the major inhibitory factors of low water activity achieved by the addition of salt and in some cases drying and the accumulation of lactic acid, a "hurdle effect" is created by a combination of other inhibitors (Table 17.9), contributing to the preservation of sausages [102].

TABLE 17.9
Factors Contributing to the Stability of Fermented Sausages

Low pH
High acidity
Accumulation of lactic acid
Various antimicrobial compounds
Hydrogen peroxide from LAB
Phenolic compounds from smoking
Low water activity
Drying
Salting
Spices
Potentially stimulate LAB, inhibit normal spoilage microflora
Low availability of oxygen
The oxygen is reduced by growth of LAB

Sausages can be split into groups based upon the extent of drying (Table 17.10). A weight loss of up to 50% can occur in shelf-stable salamis during drying [103]. Bacteria responsible for the fermentation need to be tolerant of both low water activity and salt. These environmental conditions inhibit proteolytic spoilage by gram-negative bacteria and encourage the generation of LAB, resulting in an increase in the proportions of LAB, which may even become dominant [102, 104]. Growth of LAB results in a decrease in pH and the amount of available oxygen. These inhibitory factors also contribute to the hurdle effect and in combination with other factors control the growth of the gram-negative and gram-positive pathogens commonly associated with raw meats.

Pediococcus cerevisiae, *Staphylococcus carnosus*, and *Lactobacillus plantarum* are among the most common bacteria involved in meat fermentations [101]. Natural fermentations

TABLE 17.10
Examples of Different Sausage Types

Dry Products (Moisture Content up to 35%)	SemiDry Products (Moisture Content about 50%)
Salamis	Cervelats
Pepperoni	Mettwursts
	Lebanon bologna (in US)
	Thuringer

are still used, but fermentations are increasingly initiated with starter cultures because of their greater dependability. LAB and nitrate-reducing bacteria are important members of starter cultures. Commonly used LAB include *Pediococcus cerevisiae* and *Lactobacillus plantarum*. High-salt tolerant yeasts, such as *Debaryomyces hansenii* and molds of the *Penicillium* spp. may also be included [30, 105]. Species such as *Micrococcus varians* and *Staphylococcus carnosus* are important when nitrate salts are added instead of nitrites, as they convert nitrates to nitrites, which react faster and less is required for compound stabilization [30, 49, 106].

Fermented sausages are prepared by mixing ground meat with various combinations of spices, flavorings, salt, sugar, additives, and frequently starter cultures. Common additives include acidulant, ascorbic acid, and colorings [30]. Pork, beef, mutton, or turkey meat can be used, but to achieve good sensory properties and safety, the meat must be fresh and of high quality. The meat is generally used raw, with no heat processing, as this can damage the texture of the sausage product [30]. Frequently, fermented sausages are eaten without any cooking [1]. The physical properties of the meat, especially the fat content, can affect the efficiency of the drying. To encourage efficient moisture loss, the meat particles must not be too large and the cut edges should not be effectively sealed by being covered in fat. The spices and flavoring agents modify the flavor and odor of the sausages [82]. Spices can also inhibit spoilage agents whilst stimulating lactic fermentation.

A range of times and temperatures (commonly 15–42°C) are used for the fermentation. Generally, the fermentation lasts for several days and the ripening for several weeks. During the fermentation and ripening periods, the pH falls, usually into the range pH 4–5. Investigators have demonstrated that the final pH is lower when higher fermentation temperatures are used [107].

Following fermentation, the flavor and odor of the product may be further developed by smoking and/or drying. Smoking sausages provides distinctive flavors, e.g., those of salami and hot dogs, which are developed by the accumulation of phenolic compounds from wood smoke [30]. Different wood types can be used to impart different flavors. The phenolic compounds also have antimicrobial activity and contribute to the safety and stability of the sausages.

Following fermentation, the meat is forced into casings to obtain the typical sausage shape and to provide some form of packaging. A range of properties is required for the casing materials and these are shown in Table 17.11. The casings are firmly packed to force out the air, which can cause discoloration of the meat and reduce the shelf life of the sausage.

In addition to microbial growth and the activity of endogenous meat enzymes, lipid autoxidation reactions and the breakdown of proteins to peptides and amino acids by microbial and chemical reactions are also important in generating many flavor compounds [82]. At the end of the ripening period, the flavor of unsmoked sausages can be improved by surface growth of molds, which alter the levels of amino and free fatty acids, and volatile compounds [108]. These fungi should not be toxigenic and should have proteolytic, lipolytic, and antioxidative activity [108].

TABLE 17.11
Characteristics of Appropriate Sausage Casing Material

Permeable to moisture and smoke
Acceptable to the consumer
Shrink as the meat is dried
Retain the sausage mixture
Form the required sausage shape
Edible
No detrimental effects on the sensory attributes of the products

Source: Adams and Moss [30].

The primary safety concern during sausage-making is to prevent growth of *Clostridium botulinum*. Nitrites can be used to assist the concern of *Clostridium botulinum* growth. Outbreaks of disease caused by *Staphylococcus aureus, Salmonella,* and verotoxigenic *Escherichia coli* have also been associated with fermented sausages [30]. Although counts of *Salmonella* and other Enterobacteriaceae species decline during fermentation and drying, they may not be eliminated [30, 109]; indeed, they have been shown to survive during the production of pepperoni [110].

17.5.3.3.2 Fermented Fish

Fermentation of fish is most common in Southeast Asia where fish is a major component of the human diet. Traditional and naturally produced fermented fish products such as suan yu, chouguiyu, narezushi, and plaa-som are common fermented products in countries such as China, Japan, and Thailand [111–114]. The carbohydrate content of fish is low; usually less than 1%, therefore, an additional source of carbohydrate is required for lactic fermentation. Ingredients such as rice and garlic may be added as carbohydrate sources; the carbohydrate reserve in garlic is inulin [115, 116]. The higher the level of supplemented carbohydrate, the faster the fermentation. The product is often ready after only a few weeks, making the process much more efficient. The supplementation of carbohydrates enables the fish to ferment and an acidic, stable product to be made [30].

The most common products from fish are produced by microbial fermentation and by the degrading activity of autolytic fish enzymes. These products are known as fish sauces and fish pastes. The annual production of these commodities is around 250,000 tonnes [101]. Good-quality fish sauces and pastes provide distinct aromas and flavors. They are used like condiments and are important flavoring ingredients of the diet; they are used to flavor soups, curries, salads, rice, etc.

Sauces and pastes are prepared using whole, eviscerated, or mashed fish. Low value, abundant small fish are most commonly used, often anchovies or related species. Shrimp may also be used. The fish variety, fermentation conditions, cure duration, and technique all affect the texture, amino acid content, and volatile flavor profile of the finished product. To get a product with a pleasing, fragrant aroma and taste, very fresh fish must be used.

Fish batches are washed and salted using approximately three parts fish to one part salt [54]. The salting takes the water activity below 0.75, which prevents normal fish spoilage [30]. Higher concentrations of salt slow the production rate, but extend the shelf life of the final product [105, 117]. The fish are sealed into vessels for up to 18 months or more [30]. To shorten the production time, the temperature can be increased; this can be simply achieved by placing the vessels in direct sunshine.

The proteins of the fish are broken down by autolytic enzymes producing free amino acids and volatile flavor compounds. The fish become liquefied; the liquid is harvested and any sediment is removed by filtration. The filtrate is ripened in the sun for a couple of months removing the strong fish odors. Good fish sauce is brown, clear, without sediment, has a pleasant aroma (not too fishy), and is not too salty. Fish sauce is high in protein (up to 10%), and contains all the essential amino acids. Fish sauces are also a rich source of B vitamins, especially B_{12}, pantothenic acid, riboflavin, and niacin. Other beneficial nutrients include calcium, phosphorous, iodine, and iron. Low numbers of streptococci, micrococci, staphylococci, and *Bacillus* spp. have been isolated from fish sauces and are associated with the development of flavor and aroma [54, 118]. LAB play an important role in the organoleptic properties and shelf life of these products, especially those with a lower salt content.

Fermented fish paste is prepared from salted fish with or without flavoring ingredients. It has a smoother consistency and lower moisture content than sauces. Fat is often added to the final product so that it is spreadable and can be used, for example, as a sandwich spread. The combined effects of salt, acidity, spices, and perhaps fatty acids from the fish oil of fish sauces and pastes usually guarantees microbial safety. However, these products have been implicated in outbreaks of botulism [119, 120]. A diverse collection of LAB species were found in fermented fish products including *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus reuteri*, *Pediococcus pentosaceus*, *Leuconostoc* sp., *Lactobacillus paralimentarius*, *Lactococcus garvieae*, *Lactococcus lactis*, *Lactococcus raffinolactis*, *Vagococcus* sp., *Enterococcus hermanniensis*, *Macrococcus caseolyticus*, and *Streptococcus parauberis* [111, 112, 121].

17.6 COMBINED FERMENTATIONS

The release of carbon dioxide by microorganisms has two major roles in food fermentations: (i) it can act as a leavening agent, and (ii) it can be used for carbonation of beverages. One of the most common uses of carbon dioxide is to leaven dough during bread making.

17.6.1 Bread

The use of yeasts to produce bread dates back thousands of years [122]. Breads have relatively short shelf lives, therefore the primary reason for their production was not preservation, but to improve the digestibility and eating appeal of grains. To make leavened bread, flour produced from grains, such as wheat, that contain gluten proteins is used. When the bread is kneaded, the gluten forms a matrix that makes the dough elastic and extensible [122, 124]. These properties enable the dough to stretch and retain sufficient amounts of gas to produce loaves with good volume and a fine, soft open structure [125, 126]. The gas is a combination of air incorporated during mixing and kneading and CO_2 produced from the fermentation of sugars by yeasts. The yeasts used are normally strains of *Saccharomyces cerevisiae*, commonly known as baker's yeast as they are well adapted for leavening bakery products [126]. The metabolic activity of the yeasts helps to chemically ripen the gluten enabling the dough to expand evenly and retain the gases during baking. The yeasts contribute to the flavor and provide an appealing aroma. When the loaves are baked the proteins are denatured fixing the structure and the low levels of ethanol produced by the yeasts evaporate.

To extend the shelf life of bread calcium propionate, up to a level of about 3000 ppm or ascorbic acid are added [127]. Due to the low water activity of bread the main spoilage agents are molds, particularly *Rhizopus stolonifer* and *Nerospora sitophila* [128, 129], and yeasts, which can cause the defect known as "chalky bread" [130]. In general, baked goods have a good safety record. *Bacillus subtilis* spores may naturally contaminate flour and survive baking, and subsequently germinate and grow, degrading the loaf's internal structure producing a sticky slime; this is described as "ropy bread" [131–133]. The sensory of ropy bread is not always sufficiently extreme to prevent people eating the bread and a number of *B. subtilis* outbreaks have been associated with bread [44].

Extension of the shelf life of products is one of the biggest challenges facing the baking industry today. The shelf life of bread and other baking products is short, mainly as a result of staling, which involves a number of physicochemical alterations that occur after baking and during product storage. Staling is characterized by crumb firming mainly due to retrogradation of the starch polymers and interactions between starch and proteins, crust softening due to transfer of moisture from the crumb to the crust, and finally flavor changes. These changes are responsible for the disposal of large quantities of bread (8–10%), resulting in high economic losses [134–136]. The main techniques for delaying staling and extending the shelf life are the use of hydrocolloids, emulsifiers, exogenous enzymes, etc. [134–136]. Recently, it has been proposed that a return to more traditional processes like sourdough bread making, employing pure cultures of LAB, could significantly improve bread quality in terms of delaying staling; improving taste, texture, and aroma; and generally increasing shelf life [137–139].

17.6.2 Sourdough

The use of sourdough has been established as a very important process for modern baking technology, due to the superior quality and prolonged shelf life of the sourdough baking products [140–142]. The advantages of sourdough over yeasted breads can be highlighted by its enhancement of the following features: (i) technological properties including

improved dough machinability; (ii) nutritional properties, like phytate hydrolysis; (iii) organoleptic properties such as improved bread volume, crumb texture, and unique flavor; and (iv) extended shelf life [143].

The fermentation combines the metabolic activity of LAB for souring and yeasts for leavening. Methods for their fermentation date back thousands of years [144]. The sourness of the product depends on many factors including fermentation temperature and time, type of grain, and the strains of yeast and LAB [145]. The complexity of the bread flavor is based on the lactic and acetic acids produced by LAB and flavor compounds formed by the activity of endogenous cereal enzymes, microbial metabolism, and the baking process. [146–148]. The metabolism of the LAB and yeasts also provide a range of desirable aroma products.

Traditionally, a natural starter culture that was continuously propagated from one fermentation to the next was used in sourdough fermentations [149]. The dominant yeast strain in sourdough starter cultures was classified as *Saccharomyces exiguous* [150] and later reclassified as *Candida milleri*. In San Francisco sourdough cultures the ratio of yeasts to bacteria is about 1:100. The most common LAB are members of the genus *Lactobacillus* including strains of *L. sanfranciscensis* (also referred to as *L. sanfrancisco*) [151], *L. reuteri*, *L. brevis*, and *L. pontis*. To obtain a stable symbiotic relationship the fermentation conditions must encourage metabolic activity by both the yeasts and LAB. The acid produced by the LAB lowers the pH of sourdoughs into the range pH 3.8–4.5 [54]. *Candida milleri* can tolerate this acid environment [152]. Amylases in the dough provide maltose from starch, which is utilized by the lactobacilli [153]. *Candida milleri* does not metabolize maltose but catabolizes the other sugars present, including glucose released by the lactobacilli [154]. Dead yeast cells can provide a source of fatty acids and amino acids required by the lactobacilli [155, 156]. LAB produce various compounds including organic acids that inhibit a range of mold genera, such as *Fusarium, Penicillium*, and *Aspergillus*, that are associated with bread spoilage [157]. The lactobacilli also secrete cycloheximide, which kills many organisms in the dough, but not *Candida milleri*. These act to preserve the bread.

Baked goods can be protected from fungal and yeast spoilage by destroying contaminating spores by using LAB with high and wide antimicrobial activities as starters in sourdough fermentations [158]. The microorganisms in sourdoughs that are responsible for the inhibition of the growth of certain molds are believed to be species of the *Lactobacillus* genus such as *L. plantarum, L. sanfranciscus*, *L. rhamnosus*, and *L. paracasei* [159, 160]. The antimicrobial effect of LAB is mainly related to the production of lactic and acetic acids, as well as propionic, sorbic and benzoic acids, hydrogen peroxide, diacetyl, ethanol, and phenolic and proteinaceous compounds. In addition, some strains are able to synthesize bacteriocins [161].

17.6.3 Vinegar

Vinegar is one of the oldest known culinary products [162]. It is thought that it was discovered by accident from spoilt wine; in fact it is named after the French term *vin aigre* meaning "sour wine." Vinegar is classified as a condiment that contains a minimum of 4% w/v (40g/l) acetic acid and has a pH value between pH 2.0 and pH 3.5 [30, 163]. The strength of vinegars may also be quoted in grains, with ten grains being equivalent to a concentration of 1% acetic acid [55]. Higher strength vinegars may be used for pickling; spirit vinegar is made from an alcoholic solution that has been distilled [44]. Although vinegar has been produced for thousands of years, it remains very popular. It is estimated that the annual worldwide production of vinegars is around 2000 million liters [101].

Vinegar is one of the great successes of the preservation industry, although acetic acid has numerous applications in the food industry. The shelf life of a wide range of foods is extended by storing the product submerged in vinegar; this includes pickled vegetables, such as gherkins, olives, and onions. Vinegar is also incorporated into a range of sauces and relishes such as tomato ketchup, Worcester sauce, and a variety of salad dressings and mayonnaise to improve their shelf stability. New applications of vinegars continue to be sought. One such study investigated the feasibility of incorporating acetic acid into a chitosan matrix to prepare a film that could be applied onto processed meat samples so that the acetic acid was slowly released and enhanced bacterial inhibition during vacuum packaging. Inhibition of some bacterial species was observed [164]. In addition, as new foodborne pathogens emerge, research studies focus on the tolerance of these organisms to acetic acid challenges [165, 166].

Vinegars are produced from a variety of fermentable substrates [30, 167]; fruits, honey, coconut, malt, and cereal grains are among the most common [55], but they may also be produced from alcoholic drinks such as wine or cider. Frequently the substrate used reflects the common local crops, e.g., grapes are used in France, rice in Japan, and malt vinegar is common in the UK. Vinegars are also important flavoring agents and their potential as "functional foods" is being investigated [44, 162, 167].

Vinegars are produced from a two-stage fermentation, initially an anaerobic, alcoholic fermentation of sugars by yeasts, followed by oxidation of the ethanol to acetic acid by bacteria; this second reaction is known as acetification. Acetification is also a common cause of spoilage of alcoholic beverages. Acetification can be described by the equation:

$$C_2H_5OH + O_2 = CH_3COOH + H_2O$$

Traditionally, vinegar was produced without the use of selected starter cultures (SSC), both in small as well as in large scale [168]. The process in this case relies on the indigenous yeasts strains of the sugar-rich substrates. Now, specific vinegar-making yeasts strains, usually *Saccharomyces cerevisiae* var. *ellipsoideus* [101], are often used. The alcoholic fermentation used in the production of malt vinegar essentially follows the same procedures as those used in beer making. In addition to ethanol, the fermentation also yields carbon dioxide, some higher acids, and small amounts of glycerol and acetic acid.

In contrast to alcoholic fermentation, acetification is a highly aerobic process, where bacteria oxidize ethanol to acetic acid. Small quantities of acetaldehyde, ethyl acetate, and acetoin are also produced during this reaction [101]. Commercial acetic acid bacteria are members of the genera *Acetobacter* and *Gluconobacter*. These bacteria are strict aerobes that are often found naturally in association with yeasts on plants. They are gram-negative rods and high tolerance to acid. Some acetic acid bacteria such as *A. aceti* and *A. pasteurianum* can oxidize acetic acid to carbon dioxide and water, a process known as overoxidation [101], and obviously an undesirable reaction in the production of vinegars. Overoxidation is not a problem when *Gluconobacter* species are used [162]. Also, acetic acid concentrations above 6% repress this reaction, therefore maintaining a minimum level of acetic acid during acetification reduces the risk of the acetic acid being further metabolized [30, 163, 169].

The methodology used to convert the alcoholic vinegar stock to vinegar is based on effectively combining the alcoholic stock with air and acetic acid bacteria. The rate of vinegar production is dependent on the efficiency of the aeration. Modern techniques have increased the availability of oxygen to the bacteria and enhanced the rate of vinegar production. Oxygen is not only desirable to stimulate the metabolism of the bacteria, but it is also essential for their survival. The bacteria utilize oxygen during energy production. Consequently, to avoid product failure, the equipment design must ensure a continuous, uninterrupted supply of oxygen [30]. Industrially, three main methodologies are used: (i) traditional surface methods, (ii) the trickle generator process, and (iii) the submerged method [30, 162, 169].

17.6.3.1 Traditional Surface Methods

The static surface method is the oldest production method and relies on a layer of acetic acid bacteria forming a bacterial "mat" on the surface of the vinegar stock. When the production time is long and aeration is inadequate, the product can be unpredictable, making this method less suitable for mass production. However, small quantities of traditional vinegars are still produced using these techniques for their enhanced aroma and flavor. The technique is simple and requires only basic equipment. The Orleans method, which was developed in France at the end of the 14th century [101], attempted to improve the efficiency of the surface technique by making it run semi-continuously. In the Orleans process a large wooden barrel or vat is partially filled with vinegar stock. Air holes are made just above the surface of the stock. This provides a draft of air across the bacterial film. Inlets to the barrels are covered to prevent insects and debris entering using only material that does not inhibit airflow. The barrel is left undisturbed until the required acidity is achieved. One acidification cycle usually takes about 14 days [30]. The same barrel may be used continuously for extended periods by removing volumes of the vinegar slowly from the base of the barrel and then replacing this volume with new stock, requiring acetification, into the bottom of the barrel via a tube that passes through the bacteria. Using this technique the bacterial mat remains intact and functioning, thus improves the efficiency of subsequent batches. A schematic diagram of the Orleans process for acetification is shown in Figure 17.4.

17.6.3.2 The Trickling Methods

The trickling methods are a further development of the surface technique. These processes have enhanced rates of vinegar production. This is achieved by larger areas of bacterial film in conjunction with improved aeration. The surface of the bacterial film is greatly increased by including packaging material in the process vat onto which the acetic acid bacteria form a biofilm. The material is usually inert lignocellulosic in nature, e.g., birch, vine, rattan, beechwood shavings, or corncobs, and is loosely packed. The stock is sprayed into the vat, then slowly trickles down through the packing material across the bacterial film, and at the same time air is forced up through the system from below [30]. The distance that the stock travels through the packaging and the time of contact between the bacteria and the vinegar stock can be further increased by using larger sized vats. The process is operated semicontinuously; vinegar collects at the bottom and is recirculated back through the vat until these reach the required level of acidity. This usually takes about 3–5 days, much quicker than the surface method [30, 169]. Between batches, the bacterial film remains more or less intact within the packaging material. As the oxidation of ethanol is exothermic, a cooling system is often incorporated into the vat. A schematic diagram of the quick method for acetification is shown in Figure 17.5.

17.6.3.3 Submerged Fermentation

In the 1950s, submerged culture technology began and subsequently developed to produce the most rapid rates of vinegar production. Using these systems, the time to convert alcoholic vinegar stock to vinegar is reduced to around 24–48 h [30, 162, 163]. The commercially successful processes are frequently based upon the Frings acetator [30, 162, 163]

Tiny bubbles of air are continuously sparged through the culture volume to improve the efficiency of the aeration to enable the bacteria to grow and metabolize effectively suspended throughout the culture volume. Specific strains of acetic acid bacteria have been selected for their suitability

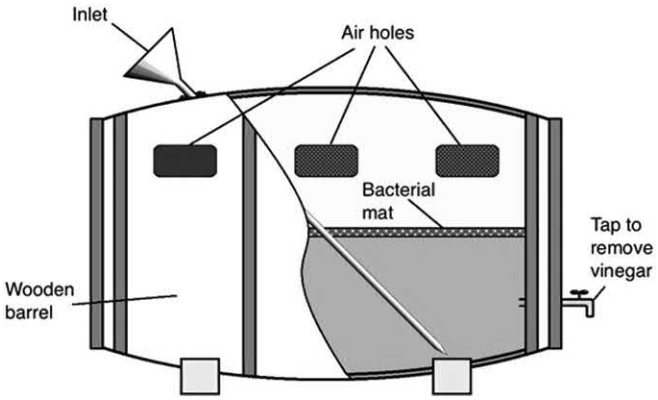

FIGURE 17.4 Schematic diagram of the Orleans process for acetification.

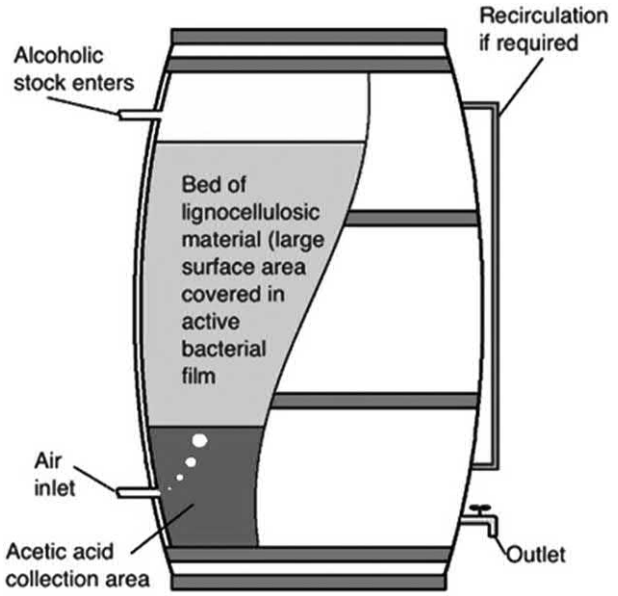

FIGURE 17.5 Schematic diagram of the quick method for acetification.

to growth in suspension. The temperature is controlled and the system is stirred continuously to maintain homogeneity throughout the culture volume. Submerged cultures are generally run automated to ensure accurate control and monitoring of the environmental parameters to prevent product loss. The systems are run semi-continuously by withdrawing only proportions of product and replacing them with equivalent volumes of fresh stock. A schematic diagram of the submerged process for acetification in vinegar production is shown in Figure 17.6. The produced flavors of vinegars using these more rapid techniques are improved by allowing a period of maturation. Before bottling, vinegars are pasteurized at 75–80°C for 30–40 seconds [101]. Defects of vinegars include cloudiness resulting from the precipitation of certain metal ions and sliminess following infection with lactic acid bacteria. Entire fermentation batches can fail if the culture becomes infected with bacteriophages.

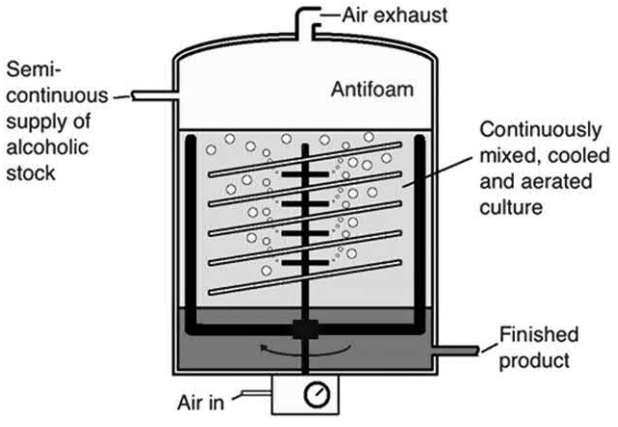

FIGURE 17.6 Schematic diagram of the submerged process for acetification in vinegar production.

17.6.4 Kefir

Kefir is produced by an acid/alcohol fermentation of pasteurized milk with a mixture of lactic acid bacteria, yeasts, and other bacteria. The final product is acidic, slightly alcoholic, liquid to semiliquid and effervescent, and is consumed as a beverage [170, 171]. Kefir grains are used to inoculate the milk. Kefir grains are comprised of proteins, polysaccharides, and a mixture of microorganisms mainly lactose-fermenting yeasts and lactic acid bacteria [172, 173]. Kefir is produced by the diverse spectrum of microbial species present. Lactobacilli are present as the largest portion (65–80%) of the microbial population [174], with *Lactococci* and yeasts making up the remaining portion of the microbes present in the Kefir grains. The yeasts consist mainly of *Candida kefir* and *Saccharomyces kefir,* while the lactic acid bacteria are comprised mainly of *Lactobacillus kefir, Leuconostoc* species, and *Lactococcus lactis.* The yeasts are responsible for the production of ethanol and carbon dioxide from lactose, the *Lactococci* produce lactate from lactose, and the lactobacilli and *Leuconostoc* species are responsible for the production of lactate, acetate/ethanol, and carbon dioxide [175, 176]. Kefir fermentation requires a moderate room temperature (17–23°C). The final composition of kefir includes 0.8% lactic acid, 1–3% alcohol, diacetyl, and acetaldehyde.

17.6.5 Oriental Fermented Products

The production of soy sauce, miso, and saki involves koji fermentation. Koji comprise soya beans or grains on which molds grow to produce enzymes such as protease, lipases, and amylases. The fungal enzymes produced digest proteins, carbohydrates, and lipids into nutrients that are used by microorganisms in subsequent fermentations. Koji is produced in many varieties depending on the products to be manufactured. Koji differ in terms of the molds, the substrate, method of preparation, and the stage of harvest.

17.6.5.1 Soy Sauce

Soy sauce is a dark brown liquid made by the fermentation of soya beans and wheat in a salt brine. The manufacture of soy sauce starts with the treatment of raw material. Soya beans, or defatted soya bean flakes, are moistened and cooked. The cooked beans are then mixed with roasted, cracked wheat in varying ratios for each type of soy sauce. The mixture is inoculated with pure culture of *Aspergillus oryzae* (*A. soyae*). After 3 days of fermentation, 17–19% of salt solution is added to the koji to produce a mash called moroni. Lactic acid bacteria such as *Pediococcus soyae* or *Lactobacillus delbrueckii* are allowed to grow on the moroni to make it acidic enough to prevent spoilage and acidic in taste. Yeasts such as S*accharomyces rouxii* and *Torulopsis* sp. grow on the moroni to produce alcohol and help the formation of flavor [177]. The moroni is aged, pressed to produce a liquid, soy sauce, which is then pasteurized.

17.6.5.2 Tempeh

Tempeh is a protein-rich food that is considered one of the world's first meat analogs. It is made by growing the mold

Rhizopus oligosporus or related species on soaked, dehulled, partially cooked soya beans, then knitting them into a firm cake, which can be sliced and deep-fried, or cut into cubes and used in place of meat in soups [12]. The processing steps of tempeh fermentation are shown in Figure 17.7. Tempeh production is not a means of improving shelf-life but it does however improve the acceptability and the nutritional quality of its raw material (soybeans).

17.7 MICROBIAL PRODUCTS AND THEIR USE AS FOOD PRESERVATIVES

As a whole, microorganisms naturally produce an arsenal of antimicrobial agents to improve their competitiveness. A common example is lactic acid, and its use as a food preservative been discussed extensively in this chapter. The major concern when substances are added to foods as preservatives is any potential risks to the consumer. Consequently, the use of antibiotics as food preservatives has not been pursued due to the health risks posed by bacteria acquiring resistance to antibiotics that are used clinically for controlling infections in humans.

The efficacy of bacteriocins produced by LAB as food additives to inhibit foodborne pathogens is of particular interest, as they are produced by food-grade organisms and could therefore be classed as "natural," and they are considered to be safe for consumers, as they have been consumed in fermented foods for generations. Bacteriocins are bactericidal peptides or proteins that are usually inhibitory to species closely related to the producer. Nisin, lactococcin, and pediocin are bacteriocins that are produced by LAB.

Nisin, which is produced by some strains of *Lactococcus lactis,* has been accepted for use as a preservative in the food industry. It is a class I bacteriocin which is active against most gram-positive bacteria including spore-formers such as *Clostridium botulinum,* which is a major concern in the food industry. Nisin is especially useful for controlling spoilage of heat-processed foods, as it inhibits the outgrowth of spores including those from *Clostridium* and *Bacillus* spp., the major spoilage agents in these foods. Nisin acts on the outside of the cell, and it destroys the integrity of the cell by creating minute holes in the cell membrane. This allows cellular components to leak from the cell and disrupts the potential across the membrane [178, 179]. Nisin is not active against gram-negative organisms, yeasts, or fungi.

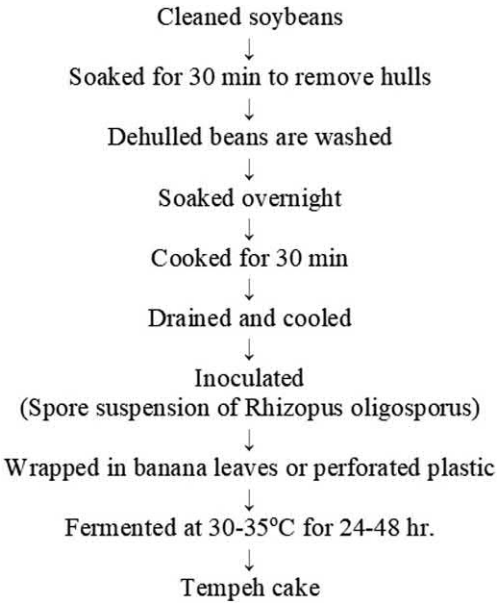

FIGURE 17.7 Flow chart of tempeh fermentation.

Nisin has been widely applied in foods as a biopreservative and pathogen inhibitory agent. Initially, nisin was used in processed cheese [180, 181]. Its application in cheese was extended to control total plate and anaerobic *Bacillus stearothermophilus*, *Bacillus cereus*, and *Bacillus subtilis* [178]

In the meat industry, nisin at 75 ppm (75 ug/g) was found superior to 150 ppm of nitrite in inhibiting outgrowth of *Clostridium sporogenes* PA3679 spores in meat slurries (182). After a 1 h exposure to 50 μg of nisin per ml and 20 mM disodium EDTA at 37°C, Stevens et al. [186] observed a 3.2 to 6.9 log-cycle reduction in population of most common species of pathogens such as *Salmonella enteritidis*, *S. typhimurium*, and *Escherichia coli* 0157:H7.

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18 Natural Antimicrobials for Food Preservation

Eddy J. Smid and Leon G. M. Gorris

CONTENTS

18.1 INTRODUCTION

The spoilage and poisoning of foods by microorganisms is a problem that is not yet under adequate control despite the range of robust preservation techniques available (e.g., freezing, sterilization, drying, preservatives). In fact, food manufacturers increasingly rely on more mild preservation techniques to comply with consumer demand for foods with a more natural appearance and nutritious quality than can be achieved by the robust techniques. In addition, consumers increasingly refuse foods prepared with preservatives of chemical origin, which still is everyday practice to achieve sufficiently long shelf life for foods and a high degree of safety with respect to foodborne pathogenic microorganisms. To meet consumer criteria, food manufacturers are searching for new, more natural alternatives that sufficiently ensure the safety of their products in the retail chain.

The search for natural alternatives to chemicals is a logical one, because nature has long been a very generous source of antimicrobial compounds, many of which play an important role in the natural defense or competition systems of living organisms (ranging from microorganisms to insects, animals, and plants). Many plants contain compounds that have some antimicrobial activity, collectively referred to here as *green chemicals*. Spices and herbs, for instance, are well known to inhibit bacteria, yeasts, and molds, and have traditionally found wide use in food preservation as well as for medicinal purposes. The use of spices and herbs or their extracts is often less effective than the use of their active ingredients, for which a number of attractive applications have been identified, as will be discussed. With respect to natural antimicrobial activity associated with microorganisms, referred to as *biopreservatives*, a mainstream field of study currently is the use of lactic acid bacteria. These bacteria have a long and safe tradition in food fermentation, and many potent applications as food preservatives have been established. The bacteriocins produced by lactic acid bacteria are especially promising, which will be discussed in detail. The use of natural antimicrobials in practice is subject to legislative requirements, which can be quite different in various parts of the world, and

this needs to be considered when discussing new development in this area of food preservation.

18.2 RATIONALE FOR THE USE OF NATURAL ANTIMICROBIAL COMPOUNDS

In many countries worldwide, there is a rapidly growing demand for environmentally friendly, safe preservatives to be used for mild food preservation. Traditional food preservation techniques have undesirable effects on the appeal of fresh food products and artificial preservatives are increasingly being banned. As a consequence, a variety of fresh or minimally processed, highly perishable vegetables have emerged on the marketplace having undergone milder preservation techniques, such as a combination of refrigeration and modified-atmosphere packaging (see Chapter 22). Mild preservation techniques can control product spoilage caused by microorganisms to some extent, mainly because they are used in adherence with the "hurdle technology" (combined processes) concept [1], as discussed in Chapter 15. However, it is now becoming more evident that potential safety hazards may occur with mild preservation systems due to the survival and growth of certain foodborne pathogens. Of special concern are cold-tolerant (psychrotrophic) pathogens, like *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*, which may grow to concerning levels during the long shelf life these perishable foods may have. Mesophyllic pathogens (i.e., *Salmonella* spp., *Staphylococcus aureus*, enteropathogenic *Escherichia coli*, and *Bacillus cereus)* pose a health hazard when temperature abuse occurs. Thus, there is an urgent need for the introduction of additional safety factors with these mild preservation techniques.

Chemical preservatives, such as sorbate and benzoate, have for long been used as reliable preservative factors to control a number of microbial hazards. However, such compounds do not satisfy the concept of "natural" and "healthy" food that consumers prefer and that the food industry, consequently, needs to manufacture. The negative reaction to chemical preservatives in our society is strongly increasing, despite the fact that such compounds are as yet indispensable in food processing. As a result, replacement of chemicals by more natural alternatives can only be relevant when necessary (i.e., when the chemical alternatives are no longer acceptable) and possible (i.e., when natural substitutes are indeed [eco-]toxicologically safe to use and effective in practice). The necessity is underlined by many in agro-industry, legislatures, and consumer organizations. The possibility is supported by many studies performed by academics and food industrialists. It is clear that natural alternatives are not always as potent as existing chemicals and that the clever use of combined processes may be a prerequisite for optimal functionality. Also, it is evident that even natural alternatives will have to pass legislative scrutiny and that the label "natural" should not be confused with inherent safety.

Nature is well known to contain many different types of antimicrobial compounds, which play an important role in the natural defense or competition systems of all kinds of living organisms, ranging from microorganisms to insects, animals, and plants. In this chapter, only natural antimicrobials from plants and microorganisms will be discussed, since these may be the most feasible substitutes for chemical food preservatives considering practical, legislative, and ethical aspects.

Regarding the development of natural antimicrobial compounds from plants (collectively called green chemicals) for food preservation, research is now focused on the potential use of phytoalexins, organic acids, and phenols. In addition, promising results have been obtained with essential oils from herbs and aromatic plants. Such essential oils consist of mixtures of esters, aldehydes, ketones, and terpenes with broad-spectrum antimicrobial activity. The toxicological basis of many herbs and spices as well as of their active components has been studied [2], and often they are known to be food-grade or even GRAS (generally recognized as safe).

With respect to the natural antimicrobial activity derived from microorganisms (referred to as biopreservatives), the most promising ongoing development in food preservation is the use of lactic acid bacteria (LAB). LAB are GRAS organisms and have a long and safe tradition in food-fermentation practices. Use of these organisms or of the antimicrobial compounds they produce has been successfully achieved in many different types of foods. Most prominently, bacteriocins produced by LAB have been under investigation worldwide for food-preservation purposes. Bacteriocins are proteins with a rather narrow antimicrobial spectrum, as compared to traditional preservatives. This apparent disadvantage is compensated for by the possibility to use these compounds for targeted control and by the fact that they are not persistent in the environment and are destroyed in the human stomach.

In the rest of this chapter, basic knowledge about the occurrence and antimicrobial properties of those natural antimicrobials of plant and microbial origin will be presented that is relevant and feasible in modern food preservation. In fact, a wealth of knowledge on the topic is available in scientific literature and elsewhere, but only a small sample will be discussed here to illustrate the ongoing quest for useful natural antimicrobials [3, 4].

18.3 NATURAL ANTIMICROBIALS OF PLANT ORIGIN

Plants have for centuries been appreciated for their antimicrobial or medicinal activity. Certain of these plants would be suitable to cultivate instead of lower value crops, thus improving cultivation revenues, which are currently under economic pressure. In many instances, antimicrobials in plants (green chemicals) function in the resistance or defense systems against microbial diseases or pests. Often, they have a particular taste or smell, which has led to them being used in the perfume and fragrance industry. Herbs and spices have been used since ancient times not only as "tastemakers," but also as preservatives or antioxidants [5–7]. A wide selection of literature exists describing the favorable properties and identifying the active components of plants.

The majority of antimicrobial plant compounds are identified as secondary metabolites, mainly being of terpenoid or phenolic biosynthetic origin. The rest are hydrolytic enzymes (glucanases, chitinases) and proteins acting specifically on membranes of invading microorganisms with antimicrobial activity [8, 9]. In general, no sharp chemical division can be made between constitutive and induced antimicrobials [10]. Based on the accumulating data on various plant compounds involved in disease resistance, Ingham [11] proposed categorizing the chemical defense systems of plants into preinfectional and postinfectional factors. Preinfectional factors are constitutive antibiotics, also called *prohibitins,* which are synthesized and stored in specialized tissues where they slow or arrest in situ microbial growth instantly upon infection. Examples of prohibitins are essential oil components with antimicrobial activity. Preinfectional factors that require a postinfectional increase in concentration for an adequate effect are called *inhibitins.* In addition, two types of postinfectional factors can be distinguished: postinhibitins and phytoalexins. Compounds belonging to the first class are toxic metabolites formed after infection by hydrolysis or oxidation of preformed compounds. The second class includes antimicrobial compounds, which are synthesized upon invasion of the host plant [12]. In this chapter, a brief overview will be given of natural antimicrobial compounds in plants that belong to one of four categories: phytoalexins, phenols, organic acids, or essential oil components.

In general, herbs and spices and several of their antimicrobial constituents are GRAS, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies. Their application in crop protection and food preservation should be facilitated by this feature, but to date plants still are a poorly exploited source of alternative antimicrobial agents. The enormous potential of plants as a source of antimicrobial compounds is well illustrated by a review of Wilkins and Board [13], who report over 1389 plants as potential green chemical sources, and more specifically by the identification of over 250 new antifungal metabolites in plants between 1982 and 1993 [10]. Obviously, not all of the potential plant sources would qualify to be introduced in our agriculture practices, simply because they would only grow in specific environments. Also, whenever a plant is considered to be exploited as a green chemical source, a thorough evaluation will have to be carried out of its value with respect to the net economy of its cultivation and actual production of the green chemical (be it the whole crop, an extract, or a purified compound), the market value of this antimicrobial preparation, and the costs for going through legislative procedures. Many of the potential sources may not pass this evaluation.

18.3.1 Phytoalexins

Phytoalexins are defined as host-synthesized, low molecular weight, broad-spectrum antimicrobial compounds whose synthesis from distant precursors is induced in plants in response to microbial infection or treatment of plant tissues with a range of naturally occurring or synthetic, artificial compounds (biotic or abiotic elicitors) [14]. More than 200 different phytoalexins have by now been identified in more than 20 plant families. Phytoalexins are broad-spectrum antibiotics, generally active against phytopathogenic fungi. In contrast to the preformed prohibitins, disease resistance due to phytoalexins is a dynamic process, requiring de novo synthesis of secondary metabolites. In addition, the enzymes responsible for the synthesis of phytoalexins are themselves synthesized in response to exposure to microbes or other effective stimuli [15]. Elicitors, the compounds triggering the synthesis of phytoalexins, range in nature from bacterial proteins [16–18] to fungal fatty acids [19] to host-plant-derived oligosaccharides [20].

The antimicrobial activity of phytoalexins is often directed against fungi [21], although activity has also been reported toward bacteria [22]. Gram-positive bacteria have been found to be more sensitive than gram-negative bacteria. Isoflavonoids, characterized by a C_6–C_3–C_6 basic skeleton structure, are among the most important chemical classes of phytoalexins [23], and studies on their application outside the natural sources have been undertaken [24]. The Leguminosae are known for the production of isoflavonoid phytoalexins, for example, pisatin (Figure 18.1a) from *Pisum sativum,* phaseollin from *Phaseolus vulgaris,* and glyceollin from *Glycine max* [25]. The production of isoflavonoid phytoalexins in plant cell and tissue cultures has aroused much interest [26, 27], and this could be a method for larger-scale artificial production when a plant itself has no sound commercial potential.

For structurally related compounds in the group of isoflavonoid phytoalexins, it was found that an increase in lipophilicity correlates positively with increased antifungal activity [28]. Terpenoid phytoalexins such as rishitin (Figure 18.1b) are mainly found in the family of *Solanaceae,* e.g., in potato tubers. The *in vitro* activity of rishitin against bacteria was found to be inhibited by low levels of the divalent cations Ca^{2+} and Mg^{2+} [29], indicating that these compounds act on the cytoplasmic membrane of the target microorganisms.

Another major group of phytoalexins, also referred to as disease- or pathogenesis-related proteins, comprises chitinases, thionins, zeamatins, thaumatins, etc. [8, 30, 31]. Some of these proteins are involved in the synthesis of other phytoalexins or phenolic compounds as constitutive or inducible enzymes. Others reportedly have a direct antimicrobial effect. Because they are proteins, they would be completely digested in the stomach and thus would have no impact on the health of a consumer. Chitinases target chitin, a major component of the cell wall of most phytopathogenic fungi and also of

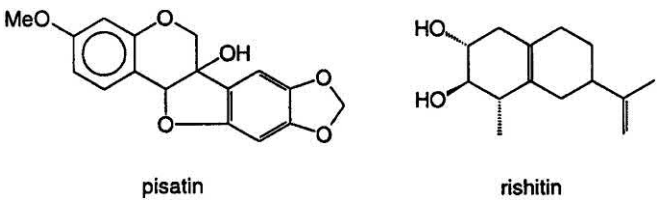

FIGURE 18.1 Phytoalexins from *Pisum sativum* (left) and *Solanum tuberosum* (right).

the skeletal structure of most invertebrates, e.g., insects and mites. Healthy plants normally contain low levels of chitinase, but their production is induced following pathogen attack. The induced chitinase accumulates either intracellularly or in the intercellular space, where their activity is required. Because vertebrates and higher plants do not contain chitin, no adverse impact is known, reinforcing the appeal of chitinases for fungal control. The use of chitinases as antifungal agents has been studied successfully in the laboratory for almost 10 years [32, 33], but practical application has not yet been realized. The same holds true for thionins, a group of small polypeptides with antifungal and antibacterial activity that occur in cereal endosperm, e.g., in barley, oat, and maize [30]. A closely related compound is viscotoxin from mistletoe. Extracts from this plant have been used against a variety of diseases and are still part of many herbal remedies.

Although the use of phytoalexins in food preservation has been suggested in many reviews [3, 34–36], there are still very few examples of the actual use of these compounds in food preservation. This is possibly due to the fact that phytoalexins in general show adequate antimicrobial effects at relatively high concentrations. In plants, this may not be a problem since these compounds accumulate locally to high concentrations, specifically in wounded plant tissue. The high concentrations necessary in food matrices when applied from an exogenous source and their occasional cytotoxicity [37] hamper the application of these compounds as food-preservative agents. The development of analogs with higher specific activity and reduced toxicity could facilitate the application of these types of compounds.

18.3.2 Organic Acids

Citric, succinic, malic, and tartaric acids are commonly found in fruits (e.g., citrus, rhubarb, grapes, pineapples) and vegetables (e.g., broccoli, carrots). Through their use as acidulants or antioxidants in foods, benefit is taken as well from their antimicrobial properties. Lactic and propionic acids do not occur naturally in foods other than in trace amounts, although they are readily formed during natural fermentation. The antimicrobial activity of the various acids is extensively documented [38]. They target cell walls, cell membranes, metabolic enzymes, protein synthesis systems, and genetic material. Thus, they are active against a wide range of microorganisms. The organic acids contained in crops may well contribute to the natural crop resistance. Many organic acids or their derivatives are already applied as food preservatives.

18.3.3 Phenolic Compounds

At the beginning of the 20th century, it was believed that plants contained compounds that were toxic toward invading fungi [39]. Initially, the abundant presence of phenolic compounds combined with their apparent *in vitro* activity toward many microorganisms was taken as an indication that these compounds could fulfill the primary role of the chemical defense system of plants. However, the role of plant phenolics in the chemical defense of plants against invading microorganisms is still unclear. Nevertheless, it has been appreciated that a vast range of phenolic compounds contribute to the defense mechanisms of plant tissues as well as to the sensory (taste, odor, appearance) and nutritional qualities of fresh or processed plants. Phenolics are characterized by an aromatic ring bearing one or, more frequently, several hydroxy substituents, including functional derivatives. Phenolic compounds usually occur conjugated, e.g., to sugars as β-D-glucopyranosides. The phenolic compounds are classified into three groups: simple phenols and phenolic acids (e.g., p-cresol, 3-ethylphenol, hydroquinone, protocatechuic, vanillic, gallic, syringic, ellagic acids), hydroxycinnamic acid derivatives (e.g., p-coumaric, caffeic, ferulic, sinapic acids), and flavonoids [36]. The latter group is the most important single group of phenolics in food, comprising catechins, proanthocyanins, anthocyanidins, and flavons, flavonols, and their glycosides. Finally, tannins, a polymeric form of phenolics, are an important group of plant phenolics, unified by the common ability to precipitate protein from aqueous solution. The antimicrobial activities of the naturally occurring phenolics from olives, tea, and coffee have been studied in more detail than those from other sources, which may in part be due to the high value of the products being processed [36]. Phenolics from spices, such as gingerol, zingerone, and capsaicin, have been found to inhibit the germination of bacterial spores. Native plant phenolics are important food-preservative factors and have, as a group, an impressive antimicrobial spectrum, although their deliberate use as food preservatives is rarely exploited.

18.3.4 Essential Oils and Their Components

Essential oils are mostly derived from spices and herbs but can also be isolated from fruits, roots, and stems of plants. Some oils and isolated plant compounds are used in food as flavoring agents. Derived from their functionality in plants, these compounds show a wide range of interesting biological activities [7]. Some compounds have been shown to attract flies for pollination, whereas others show a distinct insect-repelling activity. Others attract herbivores for seed distribution or show fungicidal or bactericidal activity to suppress infection by plant pathogenic microorganisms. Compounds that are approved for use in food and combine antimicrobial activity with low mammalian toxicity have great potential for application as natural food preservatives.

The antimicrobial activities of extracts obtained from spices, herbs, and other aromatic plants or parts thereof using organic solvents or steam distillation have been recognized for many years. Plants and plant extracts have been used since antiquity in folk medicine and food preservation, providing a range of compounds possessing pharmacological activities [40]. Most commonly, the active antimicrobial compounds are found in the essential oil fraction. With many herbs and spices, these compounds contribute to the characteristic aroma and flavor. Essential oils are mostly soluble in alcohol and to a limited extent in water. They consist of mixtures of esters, aldehydes, ketones, and terpenes [41]. Essential oil

components with a wide spectrum of antimicrobial effects include thymol from thyme and oregano, cinnamaldehyde from cinnamon, and eugenol from clove.

The impact of essential oils on bacteria, especially on pathogens, has been extensively studied in the laboratory, and significant variations have been noted. For example, *Escherichia coli* was found to be more vulnerable than *Pseudomonas fluorescens* or *Serratia marcescens* to the essential oils of sage, rosemary, cumin, caraway, clove, and thyme [42], whereas *Salmonella* Typhimurium was more sensitive to oregano and thyme oils than *Pseudomonas aeruginosa* [43]. Deans and Ritchie [44] studied the effect of 50 plant essential oils on 25 genera of bacteria and concluded that both gram-positive and gram-negative bacteria are susceptible, but the levels of impact were highly variable. Tassou and Nychas [45] have shown that the essential oil of *Pistacia lentiscus* var. *chia* (mastic gum) inhibits the growth of the food pathogen *Salmonella enteritidis* in skimmed milk. Mold growth on black table olives was found to be suppressed by methyl eugenol and the essential oil from *Echinophora sibthorpiana* [46]. The use of mustard oil in homogenized, canned beef was investigated by Drdak et al. [47]. Concentrations of 0.1% allylisothiocyanate, the active antimicrobial compound in mustard oil, did not cause unacceptable sensory effects, allowed sufficient thermosterilization, and resulted in a microbially safe product. A recent detailed review by Nychas [36] summarizes findings that essential oil compounds from many different plant sources inhibit many foodborne pathogens (Table 18.1). *Staphylococcus aureus*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *S. typhimurium*, and *Clostridium botulinum* are to some degree sensitive to extracts from linden flower, orange, lemon, grapefruit, mandarin, sage, rosemary, oregano, thyme, cinnamon, cumin, caraway, clove, thyme, allspice, mastic gum, and onion. However, most researchers inevitably came to conclude that the effectiveness of essential oils decreased when experiments were conducted in vivo. This could well be due to specific components of the food matrix, such as proteins and fats, which immobilize and inactivate the essential oil components.

The antifungal effects of essential oil components from several herbs, spices, and other plant materials have been investigated against important food spoilage or mycotoxigenic species of *Penicillium* and *Aspergillus,* but contradictory results were obtained [43, 48–51]. While some found inhibitory activity, other researchers actually noted stimulating effects. Again, the food matrix may have had a decisive influence here, and it is recommended to standardize the experimental setup accordingly.

Because essential oils contain a variety of compounds from different chemical classes, it is not possible to isolate a single mechanism by which these compounds act on microorganisms. An important common feature of essential oil components is their high degree of hydrophobicity. Therefore, these compounds partition preferentially into biological lipid bilayers as a function of their own lipophilicity and the fluidity of the membrane [52]. The accumulation of lipophilic compounds into biological membranes enhances their availability to the cell and therefore may inhibit cell vitality [53, 54]. Despite the high degree of ordering of solutes in a lipid bilayer compared with bulk liquid phase [55], a good correlation between the partitioning coefficient of various lipophilic compounds in membrane buffer systems and octanol/water two-phase systems has been observed [53, 56]. Therefore the octanol/water partitioning coefficients, which are known for many different compounds present in essential oils, can be used to assess the potential antimicrobial effect of these compounds [53]. However, the presence of specific reactive groups in compounds, the variability in membrane composition, and the metabolic capacities of the target organisms make a reliable

TABLE 18.1
Antimicrobial Spectrum of Essential Oils from Herbs, Spices, and Plants

Acetobacter sp.
Acinetobacter calcoacetica
Aeromonas hydrophila
Alcaligenes sp.
A. faecalis
Arthrobacter sp.
Aspergillus niger
A. flavus
A. ochraceus
A. parasiticus
Bacillus sp.
B. cereus
B. subtilis
Beneckea natriegens
Brevibacterium ammoniagenes
B. linens
Brochothrix thermosphacta
Campylobacter jejuni
Candida albicans
Citrobacter freundii
Clostridium botulinurn
C. perfringens
C. sporogenes
Corynebacteriurn sp.
Edwardsiella sp.
Enterobacter aerogenes
Erwinia carotovora
Escherichia coli
Flavobacteriurn suaveolens
Klebsiella pneurnoniae
Lactobacillus sp.
L. minor
L. plantarum
Leuconostoc cremoris
Listeria monocytogenes
Micrococcus sp.
M. luteus
Moraxella sp.
Mycobacterium sp.
M. phlei
Mucor sp.
Neisseria sp.
Neisseria sicca
Pediococcus sp.
Penicilliurn sp.
P. chrysogenum
P. citrinum
P. patulurn
P. roquefortii
Pityrosporum ovale
Propionibacterium acnes
Proteus vulgaris
Pseudomonas sp.
P. aeroginosa
P. clavigerum
P. fluorescens
P. fragi
Rhizopus sp.
Saccharomyces cerevisiae
Salmonella sp.
S. enteriditis
S. pullorum
S. senftenberg
S. Typhimurium
Sarcina marcescens
Staphylococcus aureus
Trichophyton mentagrophytes
Yersinia enterocolitica
Vibrio parahaemolyticus

Source: Adapted from Giraffa [72].

prediction of the toxicity of compounds based solely on their hydrophobicity difficult, if not impossible. This is exemplified by carvone and cinnamaldehyde, two compounds with comparable hydrophobicities but different antifungal mechanisms. Both compounds inhibit growth of *Penicillium hirsutum* when administered via the gas phase [57]. Full suppression of growth by carvone was observed only as long as the compound was present in the atmosphere. On the other hand, fungal growth inhibition by trans-cinnamaldehyde was found to be strictly irreversible. In conclusion, carvone acts as a fungistatic agent, whereas trans-cinnamaldehyde acts as a fungicide. The mechanism behind this difference in antifungal activity was investigated using *Saccharomyces cerevisiae* as a model organism [58]. Cinnamaldehyde was found to cause a (partial) collapse of the integrity of the cytoplasmic membrane, which leads to excessive leakage of metabolites and enzymes from the cell and finally loss of viability. In agreement with its fungistatic rather than fungicidal effect, loss of membrane integrity was not observed with carvone [58].

Considering any exploitation of essential oils, it should be stressed that large variations may occur in the yield of active compounds or total oil with the plant genotype and with different extraction methodologies, and also that variations are to be expected in the essential oil composition of the same species according to geographical location and environmental and agronomical conditions, as well as differences in essential oil content with diurnal rhythm. It is clear that essential oils or their active components are by no means a ready-to-use source from a production point of view, and many parameters need to be carefully standardized in detail in that respect.

18.3.5 Example of Application of Antimicrobials from Plants

Among the essential oil components, the volatile monoterpenes and aldehydes have attracted the recent interest of research and food industries because they can be used as food preservatives that leave a negligible amount of residues. Regarding application in practice, however, the volatile nature of the very potent compounds requires the development of suitable slow-release formulations or tailored packaging systems to maintain their functional activity for a sufficient time. For instance, with carvone, the prime monoterpene in the essential oil of caraway (*Carum carvi* L.) seeds (Figure 18.2),

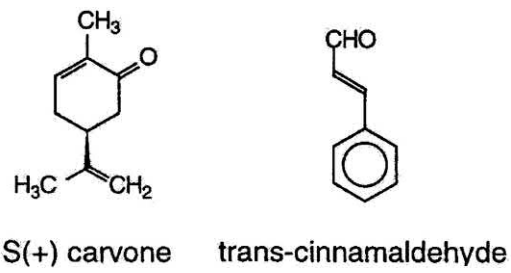

FIGURE 18.2 Structures of carvone (left) and trans-cinnamaldehyde (right), two secondary plant metabolites with antifungal activity.

a powerful antifungal effect has been found, which is already exploited for the protection of potato tubers under storage conditions [59]. However, carvone is gradually lost from the storage environment and has to be administered regularly.

Cinnamaldehyde, the major compound in cassia oil (Figure 18.2), shows potent antifungal activity against several food-associated fungi like *Penicillium* sp., *Fusarium* sp., and *Aspergillus* sp. [60]. Cinnamaldehyde also has been shown to possess antiaflatoxigenic properties [61]. When exposed to air, cinnamaldehyde is readily oxidized to cinnamic acid. Therefore, gas-phase application of this compound is less effective [57]. However, its very potent fungicidal activity and low mammalian toxicity [2] make this natural compound an interesting candidate for application as a surface disinfectant for foods. An example of the use of cinnamaldehyde in food preservation is its potential use as a surface disinfectant for tomatoes [54]. Tomatoes are particularly vulnerable to microbial spoilage at calyces and wound sites on the fruit surface. The major pathogens affecting the postharvest life of tomato fruit are *Alternaria alternata*, *Botrytis cinerea*, and *Rhizopus stolonifer.* Calyces are usually the first part of the tomato on which fungi appears. It has been shown that disinfection of tomatoes with sodium hypochlorite before packaging greatly reduced subsequent microbial spoilage. However, several countries have abandoned the use of hypochlorite for disinfection of foods, and natural plant-derived compounds with sufficient antimicrobial activity and low mammalian toxicity such as cinnamaldehyde could be good alternatives. Smid et al. [54] investigated the reduction of spoilage-associated fungi and bacteria on whole tomatoes packaged under modified-atmosphere conditions. Tomatoes were treated for 30 minutes with a solution containing 13 mM cinnamaldehyde and stored at 18°C in sealed plastic bags. Under these conditions the development of the microbial population was recorded on treated and untreated (control) tomatoes. On day 4, visible fungal growth was observed on calyces of untreated fruits. *Penicillium* sp. was found to be the dominant fungal species on the calyx. The calyx of cinnamaldehyde-treated tomatoes remained free from visible fungal growth for at least 9 days. These observations agree with the microbial analysis of the tomatoes (Figure 18.3). After 2 days of storage, pronounced growth of the bacterial population was observed on control tissues treated with 0.85% NaCl. After 4 days of storage, a significant increase in the size of the bacterial population was detected on untreated tomatoes. In contrast hardly any development of the bacterial population was detectable on cinnamaldehyde-treated tomatoes (Figure 18.3a). As expected, visible fungal growth on calyces of both untreated and NaCl-treated tomatoes appearing at day 4 corresponded with a rapid increase in size of the fungal population. The size of the fungal population on cinnamaldehyde-treated tomatoes remained small under day 11 (Figure 18.3b).

Fungicidal and bactericidal compounds from natural sources, such as cinnamaldehyde, may offer attractive possibilities for the disinfection of fresh and minimally processed fruits and vegetables. A bottleneck for practical use of these compounds may be not the efficacy, but rather specific odors

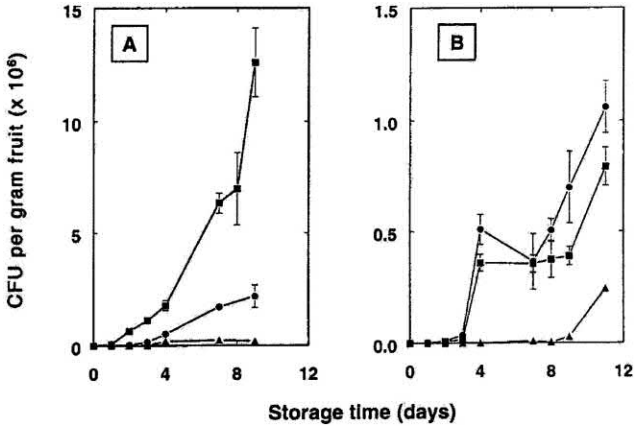

FIGURE 18.3 Development of the microbial spoilage organisms on tomatoes stored for 11 days at 18°C in sealed plastic bags. The microbial population on cinnamaldehyde-treated fruits (▲), NaCl-treated fruits (■), and untreated fruits (●) was monitored for 9 (bacteria; panel A) or 11 days (fungi; panel B). The data represent mean values of triplicate measurements, and each data point is calculated from a sample of five tomato fruits. Standard errors of the mean are indicated by error bars. (Modified from Breidt et al. [94].)

associated with such compounds at higher dosages. To overcome this problem, antifungal plant metabolites should be selected for both efficacy and minimal interference with the natural odor of the product.

18.4 NATURAL ANTIMICROBIALS OF MICROBIAL ORIGIN

Microorganisms produce a wide range of components that influence the growth of other microorganisms present in their environment. Often these components increase the competitive edge of the producing organism and as such are an important feature of their survival and proliferation. Regarding food preservation, the most important single group of organisms to be considered as a source of biopreservatives are lactic acid bacteria (LAB). These have been used in food fermentation for centuries to produce stable food products, including dairy (cheese), meat (sausages), and vegetable (sauerkraut) products. The fact that fermented products, which naturally contain these microorganisms and the antimicrobials they may produce, have been consumed traditionally without a negative health effect, has given LAB GRAS status [62]. Lactic acid bacteria may produce both antimicrobial compounds with a relatively broad inhibition spectrum (i.e., organic acids and hydrogen peroxide) as well as compounds with a rather narrow antimicrobial spectrum (i.e., bacteriocins). The use of LAB as biopreservatives is possible via the application of the producing organism as a "protective culture" to the food product and relying on its proliferation and consequent competition with the microorganisms to be suppressed. Alternatively, preparations of the active antimicrobial compounds may be utilized, with the advantage of an instant and more controllable effect. While the use of protective cultures in most countries needs only to be declared on the product, the use of antimicrobial metabolites such as the bacteriocins is subject to specific rules and regulations in food legislation. Several reviews give more detail on bacteriocins and LAB in relation to their potential for food preservation [63–70].

18.4.1 Lactic Acid Bacteria as Protective Cultures

The use of lactic acid bacteria as starter cultures in the production of fermented meats, dairy products, and vegetables is one of the oldest food-processing practices utilized and meant to stabilize food products while obtaining specific, desired sensory and organoleptic properties. The success of the fermentation process depends on the competitiveness of starter cultures, and it is exactly for this reason that LAB have been so widely used. The many different antimicrobials they produce are able to counteract a wide range of competitors that would cause problems in the fermentation process. In recent years, there has been some research into the use of LAB in food-processing applications where the outgrowth of specific problem microorganisms is to be controlled. In this case, the selected LAB are referred to as *protective cultures* and should affect pathogens or spoilage microorganisms without any negative impact on the sensory or organoleptic characteristics of the food product. Production of acids as the main antimicrobial agents is often detrimental to food quality and is not a suitable mechanism of action for protective cultures. LAB that produce a minimum amount of acids but expel bacteriocins in their environment offer good options as protective cultures.

Whereas LAB as starter cultures have become widely used and accepted, their use as protective cultures is still under development. Exploitation depends partly on legislative hurdles that relate to the consideration that protective cultures that rely for their activity on bacteriocins are intended for use as preservative agents and function by use of compounds not yet generally recognized as safe in many countries, as discussed elsewhere in this chapter. This is in sharp contrast to the legal status of starter cultures, which are considered to be processing aids or ingredients and not preservatives, and for which the mode of action seems not to be a decisive issue from the legislative point of view. Recently, it has been advocated to employ molecular biology tools to improve the performance of starter and protective cultures with regard to their production or preservation capacity [71]. Whereas, within current food legislations, natural strains already have limited access to practice, it is expected that the use of genetically modified strains will not be more easily approved.

The effort to develop protective cultures has been increasing over recent years but up to now has been confined to laboratory studies. Some review papers on the topic are available [35, 66, 69, 72–76], although several are published in sources that may not be readily accessible. A number of interesting developments with respect to the use of LAB as protective cultures for several different food categories are discussed next.

18.4.1.1 Meat Products

In a screening exercise involving 221 strains of *Lactobacillus* species evaluated for their ability to inhibit the growth of

microorganisms commonly occurring in meat products [77], a wide range of bacteria was found to be affected by individual strains, e.g., *Serratia marcescens* (by 47% of the strains), *Citrobacterfreundii* (47%), *Proteus vulgaris* (67%), *S. typhimurium* (9%), and *Brochothrix thermosphacta* (87%). In most cases, the inhibitory activity of the protective culture was attributed to lactic acid formation, although 6 of the 221 LAB isolates (all isolates of *Lactobacillus sake*) formed a bacteriocin contributing to the inhibition of *L. monocytogenes*. Experiments performed by the same researchers on comminuted cured pork (German-type fresh Mettwurst) with pH 5.7 were aimed at control of *L. monocytogenes* and showed that a strain of *L. sake* producing a suitable antilisterial bacteriocin was able to reduce the growth potential of the pathogen by about 1 log cycle [78]. A mutant of *L. sake* that did not produce the bacteriocin did not affect the number of *Listeria* inoculated into this product. In another study using *L. sake* as the protective culture, the control of *L. monocytogenes* in vacuum-packaged sliced Brühwurst (cooked sausages) was emphasized [79]. Sliced sausage samples were inoculated with a mixture of four *L. monocytogenes* serovars, fortified with either one of two bacteriocin-producing strains of *L. sake,* isolate Lb706, which produced sakacin A, and isolate Lb674, which produced sakacin 674, or of a non-bacteriocin-producing strain of *L. sake* and stored for up to 28 days at 7°C. Whereas the non-bacteriocin-producing LAB reduced counts of *L. monocytogenes* but not to an acceptable extent, both bacteriocin-producing strains of *L. sake* were able to control growth of *L. monocytogenes* adequately at the high initial counts tested.

Using bacteriocin-producing and non-bacteriocin-producing strains of *Pediococcus acidilactici* for protection of turkey summer sausages against *L. monocytogenes*, Luchansky [80] found that the pathogen could be reduced by the bacteriocin-producer by 3.4 log cycles, but by only 0.9 log cycle when the non-bacteriocin-producing strain was used. In vacuum-packaged wiener and frankfurter sausages, proliferation of *L. monocytogenes* inoculated in the products was suppressed for over 60 days by the addition of *P. acidilactici* JD1-23 at 10^7 CFU/g product, whereas the viable count of the pathogen increased from 10^4 to 10^6 in the control [81]. Degnan et al. [82] observed a clear antilisterial effect of yet another bacteriocin-producing strain of *P. acidilactici* in vacuum-packaged wieners stored at abuse temperature (25°C), where the addition of the protective culture resulted in a reduction of *L. monocytogenes* counts by 2.7 log cycles within 8 days while pathogen counts increased by 3.2 log cycles in sausages without added pediococci. In bacon, a pediocin-producing strain of *P. acidilactici* has been used in combination with reduced levels of nitrite to prevent toxin production by outgrowth of *C. botulinum* spores. Here, the protective culture would grow during conditions of temperature abuse, producing lactic acid and inhibitory pediocins. Strain *P. acidilactici* H, isolated from fermented sausage, and exhibited a broader range of bactericidal activity than any other pediococcal bacteriocin due to the production of a bacteriocin termed pediocin AcH.

Pediocin producers have also been used as protective cultures relying on their lactic acid production, rather than on the production of a bacteriocin. Hutton et al. [83] used the "Wisconsin process" (a combination of lactic acid starter culture and sucrose) to prevent toxigenesis by *C. botulinum* in reduced nitrite bacon. In chicken salads these authors found that a combination of *P. acidilactici* and glucose prevented botulinum toxigenesis. When the chicken salad was temperature abused, the protective culture catabolized available glucose to lactic acid, which caused a decrease in the pH of the product. Pathogen challenge tests verified that the rate and extent of lactic acid accumulation in the chicken salad during temperature abuse was sufficient to preclude botulinum toxigenesis.

Kotzekidou and Bloukas [84] studied the effect of protective cultures on the shelf life of sliced vacuum-packed cooked ham. They found that cooked ham produced with *Lactobacillus alimentarius* and *Staphylococcus xylosus* as protective cultures was acceptable up to 28 days, while control ham has a shelf life of 21 days. The activity of the protective cultures was directed to micrococci, staphylococci, and *B. thermosphacta*. Meat salads with relatively high pH values (pH 6.0–6.5) were studied by Hennlich and Cerny [85] for potential application of LAB as protective cultures in limiting the hygienic risks caused by food salmonellae, staphylococci, or clostridiae. The risk of pathogen growth in these foods is most apparent under temperature-abuse conditions, and the research showed that distinct cultures of lactic acid bacteria are indeed able to decrease microbial risks due to foodborne pathogens at elevated temperature. Whereas they do not reduce spoilage by bacilli, yeasts or fungi, the protective cultures used could reduce the growth of pathogens and actually spoiled the food before the pathogens could grow to hazardous levels.

Andersen [86] recently reported on a commercial protective culture developed for fresh sausages (called "FloraCarn L2"), where contamination during or after processing is a possible hazard and the protective culture can be used as an additional safety and quality factor. FloraCarn L2 was tested in fresh British sausage mince and was shown to suppress the indigenous microflora and *B. thermosphacta*. In fresh coarse-chopped sausages, the protective culture inhibited the possible development of indigenous coliform bacteria during storage.

Research on protective cultures has not always found potential positive applications for bacteriocin-producing LAB. Targeting control of *L. monocytogenes* in meats during long-term storage, Buncic et al. [87] tested *L. sake* 265 (Lb 265) and *Lactobacillus casei* 52 (Lb 52) isolated from chilled meat products as protective cultures. Although both starter cultures produced bacteriocin at 4°C, they were not able to suppress growth of *L. monocytogenes* inoculated at 10^3 CFU/g on vacuum-packaged, raw beef (pH 5.3–5.4) during 23 days storage at 4°C when they were inoculated at the same low level. The protective cultures were equally ineffective when applied to vacuum-packaged emulsion-type sausages (pH 6.4) inoculated with *L. monocytogenes* and stored at 4°C for 23 days. Apparently, the amounts of bacteriocin produced in situ

by the low initial numbers of protective cultures employed were not sufficient to inhibit or reduce *L. monocytogenes* on chilled meats to any significant extent, whereas higher initial numbers of lactic acid bacteria are not desirable in chilled meats for product quality reasons.

18.4.1.2 Fish and Seafood

Wessels and Huss [88] studied the use of protective cultures as inhibitors of *L. monocytogenes* in lightly preserved fish products. Co-culture of the pathogen with a nisin-producing strain of *Lactococcus lactis* subsp. *lactis* at 30°C resulted in a decline of the pathogen from 5×10^5 to <5 CFU/ml within 31 hours. However, when the protective culture was inoculated on slices of commercial cold-smoked salmon stored at 10°C for 21 days, no net growth was detectable. Despite this lack of evidence for in situ proliferation of the protective culture, on cold-smoked salmon slices co-inoculated with *L. monocytogenes* (10^4 CFU/g) and the protective culture (3×10^6 CFU/g), the population of the pathogen declined by a half log cycle during the first 15 days, then increased at a rate slightly lower than that of the control not inoculated with the lactococcus. Although a complete reduction of the pathogen was not achieved, the experiments proved the point that control of proliferation was feasible under practical conditions.

The use of bacteriocins from LAB for the preservation of brined shrimps, which are usually protected from microbial deterioration by addition of sorbic or benzoic acid, was tested by Einarsson and Lauzon [89]. Three different bacteriocins were evaluated (nisin Z, carnocin U149, and bavaricin A) for their biopreservative potency. With nisin Z, the most effective bacteriocin, a delay in bacterial growth was observed that resulted in an extension of the shelf life by 21 days (from 10 to 31 days). The strongest preservative effect was found with sodium benzoate and potassium sorbate, which completely inhibited microbial growth for 59 days when added to the brined shrimps at levels of 0.05–0.1% (w/w). In a recent overview paper, Huss et al. [35] presented an update on biopreservation used with fish products as they discussed a range of relevant topics: biopreservation as a full or partial alternative to salt or chemical additives, protective cultures and their characteristics, selection of protective cultures, and limitations to the application of protective cultures.

18.4.1.3 Dairy Products

To control the growth of clostridia in cheese spreads, which cause the "late blowing" of the product (a combination of gas formation and butyric acid production), Zottola et al. [90] proposed adding nisin-producing lactococci. Many clostridia are sensitive to nisin, and the use of the protective culture resulted in a significant extension of the shelf life of the product. Specifically, spoilage by *Clostridium sporogenes* was reduced in the nisin-containing cheese spreads.

Contamination by *L. monocytogenes* can also cause problems in the production of cheeses, especially in products such as the Italian cheeses Taleggio, Gorgonzola, and mozzarella, in which the pH rises during ripening and maturation. Giraffa et al. [91] showed that *Enterococcus faecium* added during the manufacture of Taleggio cheese releases a stable, antilisterial bacteriocin. An advantage of the rather narrow activity spectrum of bacteriocins from LAB is apparent from this study: the pathogen was suppressed by the added protective culture, whereas the activity of the thermophilic starter used in the cheese-making process was not affected.

Stecchini et al. [92] investigated the control of postprocess contamination of mozzarella cheeses by bacteriocin-producing strains of *Lactococcus lactis*. They observed that heat-treated cultures of such strains added to mozzarella cheese inoculated with *L. monocytogenes* and packaged in small bags resulted in a decrease in the initial counts of *Listeria*. *Listeria* counts remained significantly below those of the samples prepared without the addition of biopreservatives during a storage period of 2–3 weeks at 5°C.

Giraffa [72] presented a concise state-of-the-art review of the use of biological preservation with dairy products. In this overview, practical applications of protective cultures of LAB to increase the hygienic level of dairy products were reported as well as sensitivity of pathogens during the cheese-making process, survival of pathogens (i.e., *Listeria*) in cheese, cheese made with raw milk, and antimicrobial metabolites of LAB with emphasis on bacteriocins. The author concludes that biological preservation cannot replace good manufacturing practice (GMP) but offers an additional tool for improving food quality.

18.4.1.4 Vegetable Products

Bacteriocin-producing LAB also show potential for the biopreservation of foods of plant origin, especially minimally processed foods such as prepackaged mixed salads and fermented vegetables. Vescovo et al. [93] observed a reduction of the high initial bacterial loads of ready-to-use mixed salads when bacteriocin-producing LAB were added to the salad mixtures. Furthermore, bacteriocin-producing starter cultures may be useful in the fermentation of sauerkraut [94, 95] or olives to prevent the growth of spoilage organisms. In the fermentation of Spanish-style green olives, a bacteriocin-producing strain of *Lactobacillus plantarum* dominated the indigenous LAB without adversely affecting the organoleptic properties of the product [96]. In contrast, a non-bacteriocin-producing variant of this strain was outnumbered by the natural *Lactobacillus* population.

From studies of Cerny and Hennlich [97] on the use of LAB as protective cultures in potato salad to control food poisoning by salmonellae and toxin-producing staphylococci or clostridia, several prospects became evident. In mayonnaise-based potato salads with pH values of 5.5–6.0 that were exposed to ambient temperatures for up to one week, the protective cultures greatly reduced the hygienic risks, although they did not increase the shelf life of those products.

Hennlich [98] reported on the selection and evaluation of LAB isolated from potato salads as protective cultures for chilled delicatessen salads, assuming that they were well adapted ecologically. Important criteria for selection were minimum growth temperature, rate of acidification at refrigeration temperature, and rapid growth and acid formation at

abuse temperature (mimicking interruption of the cold chain). These criteria were adequately met by *L. casei* ILV 110 and *L. plantarum* ILV 3. When used as protective cultures (10^4 CFU/g minimum), these strains inhibited the normal spoilage flora of delicatessen salads and also suppressed growth of *E. coli* and *C. sporogenes* inoculated into meat salads during storage at chill temperature. One of the isolates, *L. plantarum* ILV 3, was found to be suitable as a protective culture for weakly acidic delicatessen salads (pH 5.0–6.0) as well.

Cerny [99] studied the inhibitory effect of a range of lactic LAB (*Leuconostoc cremoris*, *L. lactis* var. *diacetylis*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, and *Lactobacillus casei*) on the growth of several indicator microorganisms (*E. coli, Staphylococcus saprophyticus,* and *C. sporogenes*) in mayonnaise-based meat and potato salads (pH 5.5–6.5; prepared using pasteurized ingredients to eliminate endogenous LAB). It was found that addition of *L. cremoris* as a protective culture to potato salad completely controlled *E. coli* and *C. sporogenes* growth at room temp. *L. lactis* subsp. *lactis* (inoculation level 10^3–10^6 CFU/g) suppressed *E. coli* (10^2–10^4 CFU/g) in meat salad stored at room temperature. Importantly, it was concluded that the best protective effects were observed when the ratio of *L. lactis* subsp. *lactis* to *E. coli* was greater than 10:1.

18.4.2 Bacteriocins Produced by Lactic Acid Bacteria

Bacteriocins are small proteins produced by many bacterial genera, including lactic acid bacteria. Most of the bacteriocins produced by LAB inhibit the growth of other lactic acid bacteria, but some are bactericidal to a number of food pathogens and food-spoilage bacteria. In all cases, these other bacteria are gram-positive. Thus, the bacteriocins or their producers can probably not be used as a general safety hurdle, but could still be used to form a specific hurdle to suppress the growth of notorious gram-positive pathogens such as *L. monocytogenes, C. botulinum,* and *B. cereus.*

Although many different bacteriocins have currently been identified and their potential use as food preservatives is apparent, the exploitation in current practice is limited to two bacteriocins: nisin and pediocin. The limited exploitation of bacteriocins is mainly due to the rather small bactericidal range of most bacteriocins, their low efficiency of production, their limited stability in the food matrix, and, overall, their disputed regulatory status. In fact, considering the limitations to practical application, only a few of the new bacteriocins have sufficiently favorable assets in comparison to nisin and pediocin that would warrant the effort of pursuing implementation in practice. Nevertheless, despite the increasing doubt about the safety of traditional chemical preservatives such as nitrite and propionate fuels, the revival of interest seen in applied research today is aimed at the introduction of natural preservative factors such as the bacteriocins.

Ever since the identification of the inhibitory activity of a strain of *Lactococcus lactis* subsp. *lactis* in 1928, LAB have been increasingly scrutinized by bacteriocin production.

TABLE 18.2
Foods and Beverages in Which Bacteriocin Nisin Has Been Used

Food Product	Function or Use
Swiss-type cheese	Prevention of blowing faults caused by clostridia
Milk	Extension of shelf life
Tomato juice	Allows lower heat-processing requirements
Canned foods	Control of flat sour caused by thermophilic spoilage bacteria
Sauerkraut	Optimizing starter function by improving competitiveness
Beer	Inhibition of spoilage by lactic acid bacteria
Wine	Control of spoilage by lactic acid bacteria

Source: Farag et al. [42].

The inhibitory agent was later termed nisin, the first known and most extensively studied bacteriocin of LAB. Table 18.2 lists some of the potential applications of nisin. Today, more than 30 different bacteriocins produced by some 17 species of lactic acid bacteria have been identified, and much information has been obtained on the biochemistry and range of bactericidal activity. For food preservation, advantageous features of several bacteriocins are their relatively high heat resistance and inhibition of gram-positive food-borne pathogens and spoilage organisms. Much attention has been given to the inhibition of *L. monocytogenes.* This cold-tolerant bacterium, which can result in a high mortality rate, occurs in many different foods, causing problems specifically in dairy (soft cheeses) and meat (pate, sausages) products. Also, the bactericidal impact of several bacteriocins on spore-forming bacteria, such as *Bacillus* and *Clostridium* species, has been the subject of research for many decades and indicates the greater potential these bacteriocins could have in food preservation. In the following, a brief overview of the research on three interesting bacteriocins is presented with data taken from a variety of sources [65–67, 70, 77, 100–103].

18.4.2.1 Nisin

Nisin is a protein consisting of 34 amino acids, which is stable to autoclaving and effectively inhibits growth of important gram-positive foodborne pathogens like *L. monocytogenes* and *S. aureus,* and prevents outgrowth of spores of many species of *Clostridium* and *Bacillus.* It is especially active in acidic food matrices. The bacteriocin is produced by some strains of *Lactococcus lactis* subsp. *lactis*, although different strains may produce structural variants deviating slightly in exact amino acid composition. Originally nisin was considered for use as an antibiotic, but because its range of inhibition is limited, it was not judged suitable for therapeutic use. However, nisin is completely degraded in the alimentary tract and it therefore can be used safely as a food additive. Its potential use as a food preservative was first demonstrated through the successful employment of nisin-producing cultures in the

manufacture of Swiss-type cheeses. Due to their inhibition of gas-producing clostridia, blowing of the cheeses is prevented. Although vegetative cells of these organisms are killed or reduced in number by normal processing conditions, the heat-resistant spores require an excessive "botulinum cook" or the use of chemical additives to prevent their outgrowth. Nisin may be used as a natural additive to inhibit spore outgrowth or reduce their heat resistance.

Nisin has been used in conjunction with other preservative measures to enhance product safety or quality. In canned foods such as vegetables, soups, and puddings, nisin has been applied in conjunction with heating to successfully counteract heat-resistant spores of flat-sour thermophilic bacteria. Normal heating and nisin may be combined for milk production in countries where pasteurization, refrigeration, and transportation facilities are not adequate and where it is difficult to ensure the supply of good quality milk to the public. When nisin is used with acetic, lactic, or citric acid, the effectiveness of blanching and pasteurization treatments may be better than with nisin or the organic acids alone. The use of nisin in combination with nitrite in meat products has been frequently reported. Although the combined application may allow for less nitrite to exert an identical degree of inhibition of clostridia compared to nitrite alone, the meat systems seem to strongly influence the effectiveness of nisin. Inhibition of *L. monocytogenes* in raw meat, for instance, may continue for 2 weeks at 5°C, but both the inhibitory effect and the nisin-related activity rapidly diminish at room temperature. Comparable findings hold for clostridia suppression in bacon and sausages. Conceivably, binding of nisin to meat particles and high salt concentration may reduce the amount of nisin in solution where it may be active.

18.4.2.2 Pediocin

Pediocins are bacteriocins produced by LAB of the genus *Pediococcus.* The first report on pediocin production dates back to 1975, when it was found that *Pediococcus pentosaceus* inhibited growth and acid production of *Lactobacillus plantarum,* an undesirable competitor in mixed-brine cucumber fermentation. The active agent, designated as pediocin A, inhibited a broad range of LAB as well as several clostridia, *S. aureus*, and *B. cereus.* The finding implied that pediocin production might be a favorable asset of starter cultures in the fermentation of sausages and vegetables, where reported staphylococci and naturally competing LAB are the major concern, respectively.

Several applications of pediocins have been assessed with regard to food safety. Pediocin PA-1, produced by a strain of *Pediococcus acidilactici,* has been shown to inhibit growth of *L. monocytogenes* inoculated into cottage cheese, half-and-half cream, and cheese sauce for 1 week at 4°C, whereas rapid growth to high cell densities was observed in the control samples (no bacteriocin added). The activity of pediocin PA-l was not affected by fat or proteins present in the foods, while a synergistic action was noted between the effect of the bacteriocin and lactic acid. Extensive tests have shown that this pediocin is nontoxic, nonimmunogenic, and readily hydrolyzed by gastric enzymes. The potent antilisterial activity and the effectiveness of pediocin AcH and other pediocins as biopreservatives has by now been well established experimentally in beef wieners, semidry sausage, frankfurters, and fresh meat.

18.4.2.3 Sakacin

Sakacins, a group of bacteriocins produced by *Lactobacillus sake*, owe their discovery to the intensive search for natural antimicrobial compounds capable of increasing the shelf life of raw meat by inhibiting growth of meat-spoilage microorganisms and controlling *L. monocytogenes.* Several different antimicrobials are known to be produced by strains of *L. sake,* which normally reside on meat products. These strains are well adapted to the conditions in meats and conceivably are the best competitors in this food environment.

Lactocin S, produced by strain *L. sake* 45 isolated from naturally fermented sausage, is inhibitory against a range of LAB, including organisms from the same sausages. A similar bacteriocin is produced by a strain of *L. sake* isolated from Spanish thy sausages. However, the bactericidal range of this compound is much wider, including LAB and several gram-positive foodborne pathogens, e.g., *L. monocytogenes*, *S. aureus*, *C. botulinum*, or *C. sporogenes.*

18.4.2.4 Other Bacteriocins and Combined Treatments

Most other bacteriocins identified are interesting mainly from a food-quality point of view, since their bactericidal activity is directed toward closely related LAB only. The impact of this in the quality of starter cultures has been mentioned before. Bacteriocin-producing strains of *Lactobacillus helveticus* (producing helveticins and lactocins), *Lactobacillus acidophilus* (lactacins, acidophilucin), and *Lactobacillus plantarum* (plantaricins, plantacin) have been most extensively studied in this respect. With regard to food safety, the reported inhibition of *C. botulinum* and even the gram-negative *A. hydrophila* by plantacin BN-producing strains of *L. plantarum* is very noteworthy.

Several members of the genus *Carnobacterium,* a group of LAB that have been found in large numbers in chilled meat products, have been found to produce bacteriocins (carnocins) or bacteriocin-like compounds in relatively high amounts at chill temperatures, which would give them a favorable competitive edge over psychrotrophic foodborne pathogens and spoilage organisms. Again, the bactericidal range is restricted mainly to the closely related LAB, but inhibition of *L. monocytogenes* and *A. hydrophila* has been reported too.

Although gram-negative bacteria, yeasts, and molds are in general not sensitive to the action of bacteriocins from LAB, the presence of chelating agents, surfactants, or osmotic shock (high salt) may sensitize them. A combined preservation scheme would be advantageous here, as shown by Stevens et al. [104] for several combination treatments with nisin that inactivate *Salmonella* and other gram-negative species. Other reports highlighted the specific benefits of combining nisin with EDTA [105], citrate [106], lysozyme and citric acid [107], pediocin [108], and siderophores [109] in improving the

inhibitory activity of nisin toward gram-negatives or even in extending its inhibitory spectrum to cover gram-negatives.

18.4.3 Natural Occurrence of Bacteriocin Producers

Lactic acid bacteria naturally occur on many different foods (e.g., fruits and vegetables) or are used often in their production. Much research in the recent past has been devoted to tracing bacteriocin-producing LAB in fresh and fermented food products. Although the methods used were not always standardized, it is generally accepted that only a very small number of isolates obtained from food products are able to produce bacteriocins and that the spectrum of inhibition is highly variable. Vaughan et al. [110], for instance, evaluated LAB isolated from cheese, milk, meat, fruits, and vegetables for bacteriocin production targeted at a number of spoilage and pathogenic bacteria. Approximately 1000 isolates from each of the food categories were tested to inhibit *Staphylococcus aureus*, *Listeria innocua*, and *Pseudomonas fragi*. LAB isolated from cheese, milk, and meat samples inhibited *L. innocua* rather than the other target strains. LAB isolated from vegetable material generally inhibited S. *aureus*. The majority of active strains were effective against only one of the indicators, but a few were inhibitory to two or three of the target microorganisms. In our own laboratory, only 9 out of 890 isolates taken from fresh and modified-atmosphere-stored vegetables were found to be bacteriocinogenic [111, 112]. This indicates that indeed only a small part of the total population of LAB has this ability. Investigations into the capacity of different strains of *L. lactis* subsp. *lactis* present in different culture collections throughout the world to produce nisin have shown that there is drastic difference in this respect even between isolates of the same subspecies.

18.4.4 Application of Bacteriocins and Bacteriocin-Producing Cultures

Bacteriocins can be applied to food systems by three basic methods [62]. First, a pure culture of the viable bacteriocin-producing LAB can be applied, which offers an indirect way to incorporate bacteriocins in a food product. The success of this type of application depends on the ability of the bacteriocin-producing LAB to grow and produce the bacteriocin to the required extent in the food under the prevailing environmental conditions (temperature, pH, etc.). Second, a (semi-) purified preparation of the bacteriocin can be employed. In this way, the dosage of the bacteriocin can be most accurate and thus its effect most predictable. However, application of (semi-)purified preparations is limited by national regulations concerning food additives. Finally, a crude bacteriocin preparation is obtained by growing the bacteriocin-producing LAB on a complex, natural substrate (e.g., milk). This mode avoids the use of a purified compound while still being able to use a preparation of known and constant activity. This latter method is now employed for the industrial-scale production of nisin preparations. A nisin-producing LAB is grown in milk whey at optimal temperature. During the course of incubation, nisin is expelled into the substrate. At a sufficiently high level, the substrate is pasteurized, which kills the bacteria but does not affect the heat-stable nisin.

A suitable application system for natural antimicrobials of microbial origin was recently developed for the control of *L. monocytogenes* on minimally processed vegetables [111, 112]. This study set out with the concept that a suitable protective culture, in order to grow well and produce sufficient amounts of the bacteriocin, should be well adapted to the ecosystem it is used in and therefore might best be obtained from the food product considered. LAB occur on most if not all minimally processed vegetables, although they generally account for only about 1% of the natural microflora. To identify bacteriocin-producing LAB, 890 LAB isolates were obtained from different fresh and modified-atmosphere-stored vegetables and screened for their ability to produce bacteriocins [111, 112]. Only nine isolates were found that could adequately control *L. monocytogenes* on artificial growth media. Three isolates were found to have the required characteristics: one strain of *Enterococcus mundtii* and two strains of *Pediococcus parvulus*. Both types produced a bacteriocin that effectively controlled growth of *L. monocytogenes* in *in vitro* studies [111, 112]. Both pediococci, however, only produced significant amounts of bacteriocin at temperatures over 15°C and were not really suited for any application at lower temperature. The bacteriocin produced by both strains was fully identified and characterized, and appeared to be identical to pediocin PA-1, formerly only known to be produced by *Pediococcus acidilactici* [111]. *E. mundtii* produced significant amounts of a bacteriocin even at 4–10°C [113]. Thus, although it is not a LAB and does not have GRAS status, the organism is very suitable to test whether biopreservation can be the required safety hurdle for certain psychrotrophic pathogens. On laboratory media (sterile vegetable extract in agar) the application of the mundticin producer as a protective culture at 8°C was very promising (Figure 18.4a). However, on fresh, nonsterile produce, no activity was found. Either the production of mundticin on produce at low temperature is not sufficient or the mundticin is inactivated after production (enzymatic inactivation, adsorption to produce). Since the application of partially purified bacteriocin was found to significantly delay the growth of *L. monocytogenes,* the inactivation may not be the most prominent problem. Although the direct application of *E. mundtii* on mung bean sprouts was not effective in reducing the initial viable count of *L. monocytogenes* or its growth potential, a decline of 2 log units in the initial numbers was achieved when the produce was dipped in a solution of mundticin prior to contamination with the pathogen (Figure 18.4b). Identical results were obtained when the product was treated with a mundticin-containing alginate film. The increase of the viable count of the pathogen after 5 days may, again, be attributed to proteolytic degradation and growth of part of the *Listeria* population that was not affected by the intact mundticin. Noteworthy is that the counts of the pathogen did not exceed the initial inoculation level for approximately 8 days. Thus, the use of food-approved bacteriocins in a dipping

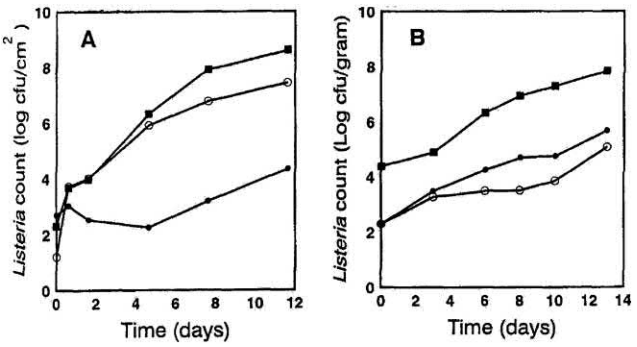

FIGURE 18.4 (A) Growth of *L. monocytogenes* (1:1 mix of strain LDCD681 and strain LDCD1087) on vegetable agar medium in the absence of *E. mundtii* (■), co-cultured with the bacteriocinogenic *E. mundtii* (●), or co-cultured with the bacteriocinogenic *E. mundtii* DSM3848 (o), using initial *E. mundtii* levels of 10^6 cfu/cm^2. Incubations were performed at 8°C under a constant flow of 1.5% O_2 and 20% CO_2, balanced with N_2. (B) Growth of *L. monocytogenes* on mung bean spouts after treatment with purified bacteriocin of *E. mundtii*. The product was dipped in sterile water (■), dipped in sterile water containing 200 BU/ml of mundticin (o), or coated with an alginate film containing 200 BU/ml mundticin (●). Again, incubations were performed under 1.5% O_2/20% CO_2/78.5% N_2 at 8°C. (Modified from Grayer and Harborne [10].)

solution or as part of an edible coating may have good potential as a biopreservative treatment for minimally processed vegetables.

18.5 LEGISLATIVE ASPECTS

Existing food legislation in most countries would not favor the use of natural compounds purified from their natural source, unless these compounds have genuinely acquired GRAS status. The purification process would bring green chemicals into the same category as synthetic chemical compounds, thus significantly lengthening the procedure for marketing approval and hampering economic implementation in practice. In fact, in most cases the legislative viewpoint on green chemicals or biopreservatives may be that they are new food additives or are applied for new purposes and consequently would require a nontoxicity record, despite their possible GRAS status. A more favorable form of application would thus be the inclusion of the spice or herb that contains the desired active ingredient or of the bacteriocin-producing strain in the food preparation because this still may be regarded as the most natural type of source.

The current regulatory status of bacteriocins and bacteriocin-producing organisms is a clear example of the current controversy between the use of the active compound or the natural source as a whole. In 1969, a joint FAO/WHO expert committee accepted nisin as a legal food additive, although it was not until 1988 that it was approved by the U.S. Food and Drug Administration (FDA) for use in certain pasteurized cheese spreads. Presently nisin is permitted in at least 50 countries for the inhibition of clostridia in cheese and canned foods. None of the other bacteriocins known to date has a fully approved legal status as a food additive, although also the application of a pediocin-producing strain of *P. acidilactici* has been approved by the U.S. Department of Agriculture (USDA) for use in reduced-nitrite bacon to aid in the prevention of botulinum toxin production by outgrowth of *C. botulinum* spores. The regulation of bacteriocin preparations from LAB stands in sharp contrast to the common use of these organisms as starter cultures. Moreover, LAB are commonly consumed in high numbers in fermented or cultured products, and are often present as indigenous contaminants in many retail products. The general conception would be that the introduction of bacteriocins in foods at levels analogous to those capable of being produced by starter cultures should be as safe as the consumption of the cultured products themselves.

18.6 FUTURE OUTLOOK

Food preservation by natural means has become a major challenge for food-manufacturing industries of all sizes and is dictated by the changes in consumers' attitudes in recent years toward chemical preservatives. All foods can be processed to extremes using physical methods that render them sterile and thus microbiologically safe. However, such foods would be unmarketable because consumers favor foods that are "natural" and "as good as fresh" because they associate such products with a healthy diet. Current research trends in food microbiology and food technology focus on mild physical preservation techniques and the use of natural antimicrobial compounds.

Food preservatives of natural origin are generally considered as potential, safe sources of antimicrobials, but their effective use in practice has been established in only a few cases. Any antimicrobial extract or purified compound from a natural source will have to undergo tough toxicological scrutiny whenever its safe use is not guaranteed by well-documented data. Toxicological data for natural antimicrobials are often lacking and are as expensive to assemble as data for chemical compounds. The economy of changing from the range of still available synthetic chemicals to green chemicals will dictate whether commercialization is feasible at all. In many countries legislation has been passed to achieve significant reductions—in some cases even a total elimination—of chemical preservatives within the next decade. As may follow from the data briefly reviewed in this chapter, research and technological development (RTD) institutions and food industries have identified a good number of possibilities for natural antimicrobials for future food preservation. However, successful marketing relies heavily on proper communication between industries, governments, and consumers. Negotiating marketing with legislative bodies up to now has been mainly on a national level, which poses a major stumbling block to the introduction of natural alternatives. The cost for industries of obtaining legislative approval for marketing are relatively high and to date have not specifically encouraged the search for natural alternatives. This increase in legislative pressure toward nonchemical strategies may favor their economic odds, but it could be even more helpful if the procedures of

approval were accelerated on a worldwide scale in favor of natural antimicrobial compounds.

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19 Antioxidants in Food Preservation

Afaf Kamal-Eldin and Jan Pokorny

CONTENTS

19.1 INTRODUCTION

Many chemical reactions proceed in foods during storage and affect their sensory quality and/or nutritional value. The main chemical reactions of interest from this point of view include fermentation, Maillard, and lipid oxidation reactions. Rancidity is used as a collective term to describe objectionable sensory attributes in foods resulting from lipid oxidation reactions [1]. In the preindustrial period, food materials were used for immediate food consumption, and food products were usually consumed within a few hours or days. The rancidity of durable products, such as nuts, flour, lard, or olive oil, was considered normal and was dealt with variably in households. With industrialization and storage of food products for days or months before consumption, processes leading to food deterioration become substantially more important. Rancidity is observed in different foods as discussed in the book *Rancidity in Foods* [1].

Rancidity is observed as objectionable flavor in, for example, stored nuts [2, 3] and biscuits [4, 5], as oxidized off-flavor in milk and milk products [6, 7], and warmed off-flavor in meat products [8, 9]. It results from the free radical oxidation of the polyunsaturated fatty acids and their interactions with proteins and amino acids in the foods [10]. Most susceptible to rancidity are essential fatty acids that do not only lose their physiological activity by autoxidation but may turn into non-nutritive agents when highly oxidized (e.g., in frying oils). Rancidity may lead to reduced levels of vitamins, such as vitamin E, vitamin C, and vitamin A or its carotene provitamins [11]. Lipid oxidation and rancidity may be inhibited by a wide range of antioxidants and synergists, as will be discussed in detail later in this chapter. Certain lipid oxidation products, such as cyclic dimers/polymers and aldehydes, may pose direct toxicity. In addition, hydroperoxides and ketones may react with primary amine groups of amino acids/proteins, and some B vitamins decreasing their biological value [2].

Research in the 20th century established the chemical basis for lipid oxidation reactions and their inhibitory mechanisms, but many details related to antioxidant protection remained paradoxical. After a breakthrough review of the problems by William Porter in 1993 [12], realization of the important role of the physical state of the oxidation reaction environment and the negative effects of increased antioxidant concentrations started to emerge. Porter observed that polar antioxidants are more active in oily environments, whereas non-polar antioxidants are more active in watery emulsions. In the same year, Ulla Brimberg performed kinetic analysis of lipid oxidation data in different media and concluded that lipid oxidation is catalyzed by hydroperoxide-loaded micelles [12–15]. Unfortunately, her papers were not noticed, possibly because her work was presented in a non-conventional way. In 1994, Edwin Frankel and coworkers suggested the interfacial phenomenon as an explanation of the polar paradox, but considered the interface as that between oil and air [16, 17]. Becker, Ntouma, and Skibsted [18] and the group of Eric Dekker [19–24] further refined the interfacial concept, considered the involvement of association colloids, and highlighted the importance of colloidal interfaces. Berton-Carabin et al. [25] reviewed the involvement of the interfacial layer in the oxidation of oil-in-water emulsions. Thus, lipid oxidation follows the roles of supramolecular chemistry where molecular organization is a decisive factor for stabilization by antioxidants [26]. With this paradigm shift in our understanding [27], new possibilities for food preservation technologies are emerging.

19.2 TYPES OF RANCIDITY IN FOODS

Rancidity describes the objectionable defects in food quality manifested as changes in odor or flavor leading to a significant deterioration of the sensory quality that might lead to rejection of the food [1]. Rancidity may include several types of reactions, but the degradation due to changes in lipid components is considered the main feature of rancidification [1]. The most important types of rancidity are summarized in Table 19.1.

Lipolytic rancidity is mainly caused by the cleaving action of lipases (triacylglycerol-acyl hydrolases, EC 3.1.1.3) on triacylglycerols to yield free fatty acids and partial glycerol esters, i.e., mono- and diacylglycerols. The release of small amounts of free fatty acids is often not perceptible by human senses as flavor deterioration, but release of higher amounts may be perceived as sourness, e.g., in crude vegetable oils. Milk fat is one exception because small amounts of free butyric acid impart a typical disagreeable off-flavor such as that encountered in rancid butter [28]. Similarly, caproic, caprylic, and capric acids released by the lipolysis of seed oils of palms (Palmaceae, such as coconut or palm kernel oils) result in a soapy off-flavor. The soapy flavor, frequently observed in stored food products containing coconut, is not only due to soap but to all derivatives (free fatty acids, methyl or ethyl esters) having a hydrocarbon chain of 6–10 carbon atoms. There is a link between lipolytic and oxidative rancidity because free fatty acids have higher oxidation rates than esterified fatty acids [29, 30].

Oxidative rancidity, resulting from the oxidation of unsaturated fatty acids, is the most important type of food rancidity that is inhibited by antioxidants. The primary oxidation products are typically lipid hydroperoxides, which degrade to low and high molecular weight secondary oxidation products. Volatile oxidation products possessing 3–12 carbon atoms, including aldehydes, ketones, alcohols, short-chain acids, and hydrocarbons, are responsible for the sensory perception of rancidity. A very low level of rancidity is not perceived by most consumers and it might sometimes make the flavor richer and more acceptable. Virgin olive oil or frying oils are typical examples of products where minute amounts of oxidation products are desirable but higher amounts are objectionable. Rancidity is a very complex phenomenon, and rancid foods may be described as, for example, old, warmed-over, cardboard, wet dog, or dumpy. Small amounts of oxidation products of polyunsaturated fatty acids impart a fried flavor to food [31]. Higher amounts of the same compounds may cause the perception of burned flavor. Rancidity may be controlled by decreasing the storage temperature, the access of oxygen, and the degree of unsaturation of the lipid fraction. When application of the aforementioned methods is not possible or satisfactory, the best way to control rancidity is with the addition of antioxidants. The oxidative rancidity is the central topic of this chapter, which discusses its mechanisms, implications, and inhibition by antioxidants.

Flavor reversion, typical for soybean oil, is a type of rancidity that appears during storage of fully refined, bland soybean oils and imparts a "beany" off-flavor to the product. It results after minute absorption of singlet oxygen by the oil leading to the formation of specific oxidation products containing furan groups. Ketonic rancidity, with a characteristic floral off-flavor, is sometimes observed during the storage of foods containing short- or medium-chain (4–10 carbon atoms) fatty acids, such as those containing milk fat or coconut oil [32]. Ketonic rancidity is caused by microbial oxidation of medium-chain fatty acids into the respective alkan-2-ones or methyl ketones [33]. This flavor in objectionable in butter, but it is characteristic of blue cheese aroma.

19.3 KINETICS AND MECHANISMS OF OXIDATIVE RANCIDITY

Lipid oxidation involves the deteriorative change of polyunsaturated fatty acids and other unsaturated lipid substrates (e.g., sterols, carotenoids) into hydroperoxides and the subsequent decomposition of these hydroperoxides into a wide range of secondary oxidation products [34, 35].

$$LH + O_2 \rightarrow LOOH \tag{19.1}$$

$$LOOH \rightarrow \text{Secondary oxidation products} \tag{19.2}$$

The oxidation of LH by oxygen, called autoxidation, is a free radical chain reaction consisting of three main phases,

TABLE 19.1
Types of Rancidity Occurring in Fats, Oils, and Fatty Acids

Types of Rancidity	Main Substances Producing Rancidity	Types of Chemical Reaction	Foods Subject to This Type of Rancidity
Lipolytic	Low fatty acids, medium-chain fatty acids	Enzymic hydrolysis	Milk fat, palm seed oils
Oxidative	Lower aldehydes and ketones	Autoxidation, enzymic oxidation	Foods containing polyunsaturated fatty acids
Flavor reversion	Oxygen-substituted cleavage and rearrangement products	Oxidation, cleavage, and rearrangement	Soybean oil
Ketonic	2-Alkanones (methyl ketones)	β-Oxidation and enzymic decarboxylation	Milk fat, palm seed oils

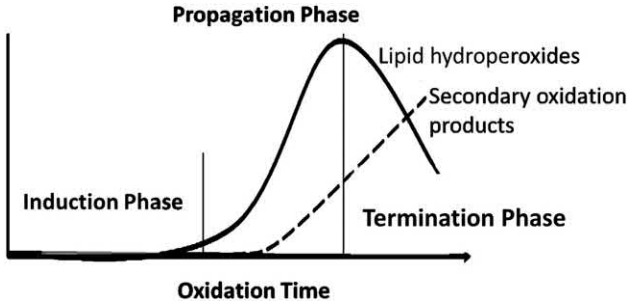

FIGURE 19.1 Typical lipid oxidation scheme for the evolution of hydroperoxides and secondary oxidation products consisting of three phases: initiation, propagation, and termination. The x-axis is the oxidation time and the y-axis is any indicator of change related to a concerned product.

namely, initiation, propagation, and termination [36, 37]. A typical kinetic curve describing the lipid oxidation cascade is shown in Figure 19.1.

19.3.1 The Initiation Phase or Induction Period

The initiation of the lipid oxidation reaction is not yet exactly understood, but depending on the catalyst(s) involved, oxidation is slow during an induction period (or a lag phase). During this stage, the reaction rate follows a first or pseudo-first order with respect to lipid hydroperoxides. It is assumed that lipid oxidation is initiated by a *discrete reaction* between an unsaturated lipid and an oxidant ($X^{\bullet}$) leading to the formation of the first alkyl radicals ($L^{\bullet}$) that immediately unite with oxygen to form hydroperoxyl radicals:

$$LH + X^{\bullet} \rightarrow L^{\bullet} + XH \quad (19.3)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (19.4)$$

A hydrogen atom from the methylene group between two double bonds (*bis-allylic hydrogen*, -CH=CH-C**H**$_2$-CH=CH-), as is present in linoleic and linolenic acids, is the primary site of dehydrogenation [36–38]. In monoenoic fatty acids, such as oleate, free radicals are formed by the abstraction of a hydrogen atom on either side of the double bond (allylic hydrogen, -C**H**$_2$-CH=CH-C**H**$_2$-) but the activation energy of this reaction is much higher than that required for the abstraction of a bis-allylic hydrogen. Initiation reactions are catalyzed by temperature, initial presence of hydroperoxides, transition metal ions, and lipoxygenase-type enzymes.

Several isoenzyme lipoxygenases (linoleate oxidoreductases, E.C. 1.13.11.12) and lipoxygenase-like activities in foods directly catalyze the oxidation of linoleic, linolenic, and structurally related fatty acids and produce hydroperoxides with specific stereo- and positional selectivity by Reaction 19.1 [39, 40]. In foods and other biological materials, lipoxygenases are commonly accompanied by respective lyases, which cleave hydroperoxides into different low molecular weight compounds. The presence of transition metal ions catalyzes the decomposition of hydroperoxides into two free radicals that will initiate further reaction chains. Therefore, traces of copper and iron, and to lesser degree manganese and cobalt, are important promoters of lipid oxidation.

It has been accepted that the end of the induction period is reached when the antioxidant concentration is diminished and the quantity of formed hydroperoxides reaches a critical micelle concentration (cmc) dependent on the composition and characteristics of the lipid milieu [13–15, 41, 42]. When this cmc is reached, there is enough concentration of lipid hydroperoxide molecules to promote oxidation by degenerate branching with lipids (monomolecular) or between themselves (bimolecular):

$$LOOH + LH \rightarrow LOO^{\bullet} + L^{\bullet} + H_2O \quad (\text{Monomolecular}) \quad (19.5)$$

$$LOOH + LOOH \rightarrow LOO^{\bullet} + LO^{\bullet} + H_2O \quad (\text{Bimolecular}) \quad (19.6)$$

The bimolecular reactions predominate at the end of the induction period. The peroxyl and alkoxyl free radicals formed by these degenerate branching of lipid hydroperoxide molecules react with unsaturated fatty acids initiating new reaction chains. The duration of the induction period is determined by the degree of unsaturation of lipid substrates, the temperature, and the presence and nature of prooxidants, primary antioxidants (phenolic compounds), and a wide range of antioxidant synergists, as will be discussed later.

Fats, oils, and lipid-rich foods, which turn rancid by oxidation, are mostly oxidized by air oxygen, which penetrates foods and is dissolved in both aqueous and lipid phases. If the reaction is catalyzed by enzymes, the oxidant is still air oxygen. Other oxidants (Table 19.2) are of minor importance. In the presence of photosensibilizers (such as chlorophylls) and light, ordinary triplet oxygen is converted to a singlet oxygen (Figure 19.2), which is 100–300 times more reactive [43, 44]. A singlet oxygen molecule is directly added to a double bond of unsaturated lipids to produce hydroperoxides that are easily cleaved to produce initiating free radicals. Carotenoids are efficient quenchers of singlet oxygen, and with this mechanism, these can synergize the effect of tocopherols under low temperatures and low oxygen pressure [45].

19.3.2 Propagation Phase

Peroxyl radicals propagate chain reactions by abstracting bis-allylic or allylic hydrogen atoms from polyunsaturated fatty acids forming a hydroperoxide and an alkyl free radical,

TABLE 19.2
Important Oxidants in Food Products

Oxidant	Importance	Occurrence
Air (triplet) oxygen (autoxidation)	Most important in processed and stored foods	General
Enzymatically catalyzed oxidation	Stored raw materials	Oilseeds, nuts, cereals legumes
Singlet oxygen	In light and presence of sensitizers	Edible oils, green foods
Ozone	Very low in foods	Essential oils
Quinones	In foods subject to enzymic browning	Fruits, vegetables, potatoes
Metals	Initiation of free radical oxidation	Meat, fruits
Superoxide anion	Mainly in in vivo systems	Meat
Hydrogen peroxide	In presence of ascorbic acid	Fruits, vegetables
Lipid hydroperoxides	In presence of polyunsaturated acids and carotenoids	Fruits, vegetables, fatty foods

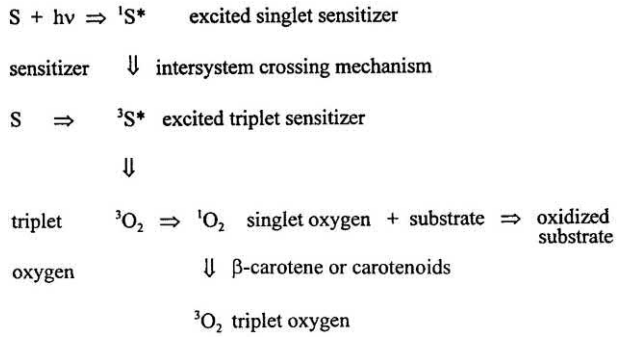

FIGURE 19.2 Generation of singlet oxygen and its quenching during photooxidation.

which easily absorb oxygen forming new peroxyl radicals by Reaction 19.4:

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \tag{19.7}$$

This reaction sequence is repeated several times, therefore, it is called autocatalytic or chain reaction. During this reaction, the double-bond system of the original polyunsaturated fatty acid is usually isomerized into a more stable conjugated diene system. The alkoxyl radicals formed by Reaction 19.6 or by the decomposition of hydroperoxides may rearrange to epoxy alkyl radicals leading to epoxy derivatives [46, 47]:

$$LO^{\bullet} + O_2 \rightarrow (O)LOO^{\bullet} \tag{19.8}$$

During the propagation phase, the unsaturated fatty acid substrates decline and hydroperoxides are formed exponentially. This is accompanied by a significant increase in the concentrations of secondary oxidation products including long-chain hydroxy- and keto- derivatives as well as breakdown products including hydrocarbons, alcohols, aldehydes, ketones, and acids. Hydroperoxides of polyunsaturated fatty acids have no particular odor or flavor, but their decomposition volatile low molecular weight products (3–12 carbon atoms) impart the rancid off-flavor to oxidized fats and oils [48]. Those secondary oxidation products are easily oxidized in turn giving rise to lower molecular weight fatty acids and other tertiary reaction products.

19.3.3 Termination Phase

When the concentration of unsaturated lipid substrates is diminished, the chain of the propagation reaction is interrupted by the recombination of free radicals leading to the last stage of autoxidation, i.e., the termination reaction.

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{non-radical secondary oxidation products} \tag{19.9}$$

$$LOO^{\bullet} + LO^{\bullet} \rightarrow \text{non-radical secondary oxidation products} \tag{19.10}$$

$$LO^{\bullet} + LO^{\bullet} \rightarrow \text{non-radical secondary oxidation products} \tag{19.11}$$

Lipid polymers, mainly dimers and oligomers, are produced besides the cleavage products, but these do not substantially affect the sensory value. Diperoxides are also formed by secondary oxidation of hydroperoxides, especially in advanced reaction stages of polyunsaturated lipids at sufficient access of oxygen.

19.4 ANTIOXIDANTS AND THEIR MECHANISMS OF ACTION

In the broad sense, antioxidants (or oxidation inhibitors) are substances that can protect food and other biological materials from autoxidation by scavenging free radicals [49]. In the presence of antioxidants, the induction period is significantly prolonged as exemplified in Figure 19.3. Antioxidants may also inhibit the decomposition of lipid hydroperoxides that would, otherwise, act as free radical chain initiators [50]. In this chapter, the broader sense is applied. The most important types of antioxidants are summarized in Table 19.3. Phenolic antioxidants and their synergists are the most important representatives of these compounds in food applications [51].

Phenolic antioxidants (AH) exert their protective effects by reacting with peroxyl radicals produced in oxidized lipids forming a hydroperoxide molecule and the free radical of the antioxidant [36, 37]. Antioxidant radicals are relatively stable so that the back reaction is extremely slow. They do not initiate a chain autoxidation reaction unless present in a very large excess. They react in a similar way with an alkoxyl free radical formed during the decomposition of hydroperoxides [52].

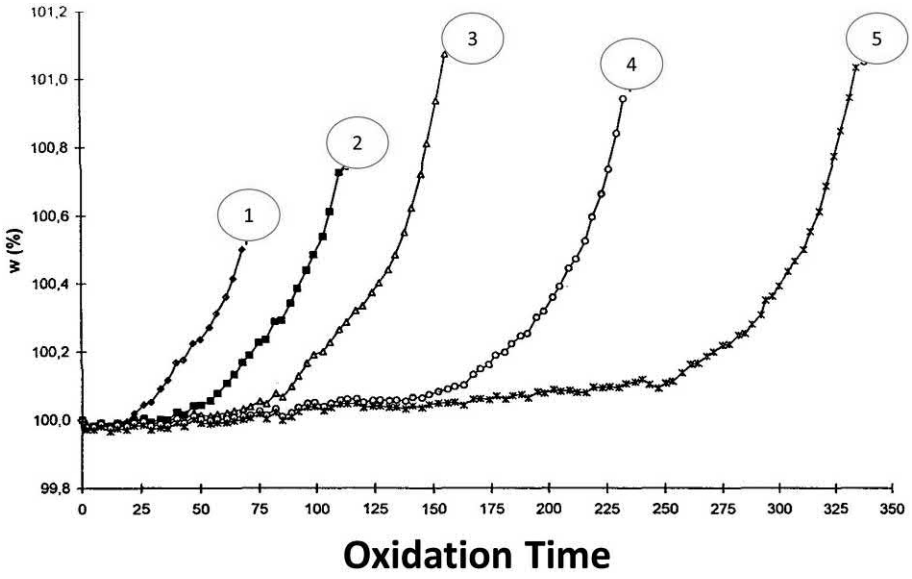

FIGURE 19.3 The course of oxidation reaction during storage of stabilized rapeseed oil stabilized with hexane rosemary extract under conditions of the Schaal oven test at 40°C. The x-axis is the storage time and the y-axis is the percent increase in weight. Curves: (1) control (without antioxidants), (2) 0.01% extract, (3) 0.02% extract, (4) 0.05% extract, and (5) 0.1% extract.

TABLE 19.3
Types of Oxidation Inhibitors (Antioxidants and Synergists)

Group of Compounds	Mechanism of Action	Example of Inhibitors
Antioxidants	Scavenging of free radicals	Tocopherols, phenolic compounds
Chelating agent	Binding heavy metals into inactive complexes	Polyphosphates, citric acid, tartaric acid, EDTA
Singlet oxygen quenchers	Quenching of singlet oxygen and its transformation into triplet oxygen	Carotenoids
Hydroperoxide deactivators	Reaction with hydroperoxides	Cysteine, selenometathione, amino acids, proteins, protein hydrolysates
Antioxidant regenerators	Regeneration of an antioxidants from their radicals	Ascorbates
Surfactants	Stabilize hydroperoxide-loaded micelles	Phospholipids

Antioxidant radicals also react with other lipid hydroperoxyl radicals by combination.

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet} \quad (19.12)$$

$$LOO^{\bullet} + A^{\bullet} \rightarrow LOOA \quad (19.13)$$

Antioxidants may also stabilize lipid hydroperoxides and inhibit their decomposition into free radicals by the formation of physical bonds [50, 53]. It was shown that the presence of antioxidants, such as α-tocopherol, extends the induction period by increasing the critical micelle concentration necessary for the transition from the induction period to the propagation phase [13–15, 41, 42]. Antioxidant dimers, and even trimers/oligomers, may be formed and contribute a modest antioxidant activity of their own. Antioxidant radicals may react with a molecule of oxygen to form an antioxidant peroxyl radical, especially when both the antioxidant(s) and oxygen are present in excess. Free antioxidant radicals can also react with some labile compounds, such as terpenes, which form easily free radicals. Changes of antioxidants occur under conditions of food processing and storage, including culinary meal preparation [54].

Usually, there is an optimum antioxidant concentration for maximum stabilization of lipids [38]. When the antioxidant is present at higher concentrations than this optimum, the antioxidant and/or its radical(s) may participate in side reactions leading to loss of antioxidant efficacy [55, 56]. This loss of efficacy is manifested as an increase in the oxidation reaction rate during the induction period (increased rate of initiation) as well as faster rate of antioxidant consumption. Although the induction period might be longer due to the high antioxidant concentration, the level of hydroperoxides during the induction period is higher (Figure 19.4).

Kinetic modeling of the oxidation of methyl linoleate in the presence of α-tocopherol including 53 individual reactions was employed to reveal the reactions involved in the peroxidative effect of high concentrations of the antioxidant [57]. At least, three different reactions contributed significantly to this effect of the antioxidant:

1. Reduction of hydroperoxides by the antioxidant molecule leading to the generation of alkoxyl and antioxidant radicals:

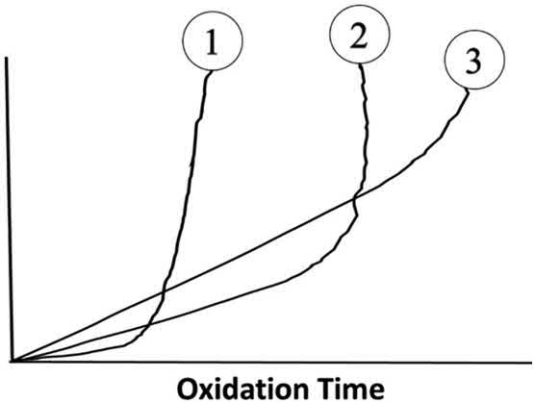

FIGURE 19.4 The effect of antioxidant concentration on its activity during the oxidation of vegetable oils. The x-axis is the storage time and the y-axis is any indicator of change related to hydroperoxide formation. Curves: (1) low antioxidant concentration (e.g., 100 ppm in sunflower oil), (2) intermediate antioxidant concentration (e.g., 500 ppm in sunflower oil), and (3) high antioxidant concentration (e.g., 1000 ppm in sunflower oil). This phenomenon is called loss of antioxidant efficacy and it is very important during the storage of foods.

$$AH + LOOH \rightarrow A^{\bullet} + LO^{\bullet} + H_2O \quad (19.14)$$

2. Abstraction of the hydrogen atom from the methyl linoleate molecule and from the methyl linoleate hydroperoxides by the tocopheroxyl radical suggested by Mukai et al. [58]:

$$A^{\bullet} + LH \rightarrow AH + L^{\bullet} \quad (19.15)$$

$$A^{\bullet} + LOOH \rightarrow AH + LOO^{\bullet} \quad (19.16)$$

3. The homolytic decomposition of quinolide peroxides, which are the combination products of the antioxidant and lipid peroxyl radicals (LOOA) formed in Reaction 19.17 to generate alkoxyl radicals:

$$LOOA \rightarrow LO^{\bullet} + AO^{\bullet} \quad (19.17)$$

The loss of tocopherol antioxidant efficacy can be manifested to a higher degree when oxidation experiments are performed at a lower temperature, i.e., at a low initiation rate [59]. It has been observed that the inhibitory effect of antioxidants on lipid oxidation reactions may be significantly potentiated and the loss of antioxidant efficacy counteracted by the presence of the antioxidant synergists. Synergists are substances that have no antioxidant activity of their own but they can increase the activity of an antioxidant. Antioxidant synergists substantiate the inhibitory effect of antioxidants via different mechanisms.

Metal ions of transient valency (such as copper, iron, cobalt, chromium, or manganese) are very active prooxidants not only in their ionic forms but also as complexes, e.g., heme derivatives and metalloproteins [60–62]. Therefore, substances that are able to bind these metals into inactive complexes can inhibit oxidation reactions of lipids. Many metal-chelating substances are present in foods as natural components, especially in plant materials. The most frequently used metal-chelating synergists include polyvalent inorganic (phosphoric acid) and organic acids (e.g., ascorbic acid and its isomer erythorbic acid or isoascorbic acid, citric and tartaric acids, and different amino acids). In order to increase its solubility in fats and oils, ascorbic acid is often esterified with higher fatty acids, such as palmitic acid. Ascorbyl palmitate is stable against oxidation on storage, but ascorbyl oleate would be better soluble in oils. Citric acid is also added as an ester, e.g., isopropyl ester. Ascorbyl palmitate is active as a synergist to tocopherols in vegetable oils and lard [63]. Citric and ascorbic acids were active inhibitors of rancidity in frozen horse mackerel, where they probably act as metal chelators [64]. Polyphosphates are added to inactivate iron in food, especially in various meat products [65]. Even some phenolic antioxidants may bind metals into complexes, e.g., quercetin. Silicon oil is often added to frying oils to inhibit degradation by protecting against the diffusion of air oxygen; the activity is, however, at least partially, attributed to prevention of dissolution of iron in oil during frying [63]. Phytates (salts of phytic acid, *myo*-inositol hexaphosphate) and oxalates are also common representatives of this group. The chelating activity depends on the pH value, water content, and other factors of the medium. Usually, metals are not completely inactivated by any agent; only their prooxidant activities decrease by at least a partial chelation. We have shown in phosphatidic acids and in pheophytins that the chelating activity is often very high. Nevertheless, it should be kept in mind that even minute traces of free metals (a small fraction not bound in complexes) are sufficient in some cases to promote autoxidation efficiently.

Singlet oxygen, which may be formed from ordinary triplet oxygen by action of light in the presence of a photosensitizer such as chlorophylls and pheophytins (Figure 19.2) or by the Russel mechanism, is a very reactive oxidant. In the presence of high concentrations of photosensitizers, the initiation rate is extremely high:

$$\underset{}{L_1OO^{\bullet} + L_2OO^{\bullet} \rightarrow} \underset{\text{Tetoxide}}{L_1OOOOL_2} \rightarrow \underset{\text{Alcohol}}{L_1OH} + \underset{\text{Ketodiene}}{L_2{=}O} + \underset{\text{Singlet oxygen}}{{}^1O_2} \quad (19.18)$$

Various substances are effective from this standpoint but in food materials, carotene and other carotenoids are the most important singlet oxygen quenchers [66]. Oxygenated carotenoids, or xanthophylls, have similar deactivating activity, even when they have no provitamin activity like that of carotenes. Tocopherols increase the activity of carotenes in this respect [67]. The structurally related vitamin A has a similar activity, but it is usually present in much smaller quantities in foodstuffs than are carotenoids. Ascorbyl palmitate is an active singlet oxygen quencher too [68]. In the presence of tocopherols, carotenoids may also prohibit low-degree

oxidation by adding free radicals and acting as radical sink because of their extended conjugated double bonds:

$$\text{Car} + (\text{LOO}^{\bullet})_n \rightarrow \text{Car-}\left(\text{LOO}\right)_n^{\bullet} \quad (19.19)$$

However, carotenoids would act as prooxidants in a rich oxygen environment [69]. Other synergists, particularly proteins, peptides, and amino acids, may operate by reducing lipid hydroperoxides into less reactive hydroxyl derivatives without the involvement of alkyl radicals [70]. Sulfur compounds are very active substances, which are mostly bound in proteins. Thiols, such as cysteine, are oxidized into cystine, which belongs to disulfides. Cystine may be further oxidized by another molecule of a hydroperoxide into sulfinic acid. Similarly sulfides, such as methionine, react with hydroperoxides, forming sulfoxides. This reaction product may react with another molecule of a hydroperoxide, resulting in the respective sulfone. Onion and garlic contain sulfur compounds possessing hydroperoxide-decomposing activity [71, 72].

$$2\text{R-SH} + \text{LOOH} \rightarrow \text{R-S-S-R} + \text{LOH} + \text{H}_2\text{O} \quad (19.20)$$

$$\text{R-S-S-R} + \text{LOOH} \rightarrow \text{R-S-O-L} + \text{R-S-OH} \quad (19.21)$$

$$\text{R-NH}_2 + \text{LOOH} \rightarrow \text{R-NH-O-L} + \text{H}_2\text{O} \quad (19.22)$$

Selenium-containing amino acids (selenocysteine or selenomethionine) present in traces in natural proteins may react in a similar way and help to retain vitamin E in the body [73]. Selenium has more importance as part of an antioxidative enzyme, selenoglutathione oxidase, inactivating free radicals and other oxidants, particularly hydrogen peroxide. In addition, lipid hydroperoxides may react with free amine groups of proteins; imines are formed from the intermediary products by subsequent dehydration. Various basic groups, such as histamine or indole, can deactivate hydroperoxides following similar mechanisms. Carnosine, a histidine dipeptide, was found to be efficient in decomposing hydroperoxides [74]. Many amino compounds can deactivate free radicals, therefore, these amino derivatives have an anticarcinogenic activity. Free amino acids are present in protein hydrolysates, which are thus active, e.g., in various meat products [75].

Ascorbic acid is a strong reducing agent (mv) capable of regenerating the tocopherol molecule (TOH) from its radical (TO•) [76].

$$\text{Ascorbates} + 2\text{TO}^{\bullet} \rightarrow \text{Dehydroascorbates} + 2\text{TOH} \quad (19.23)$$

Ubiquinine [77] and green tea catechins [78] were also shown to recycle tocopherols in a similar way to ascorbate [79]. As mentioned earlier, it has become clear that lipid oxidation reactions are highly influenced by the molecular organization of the original unsaturated lipids and the emerging hydroperoxides and other polar derivatives [12–27]. As the concentration of hydroperoxides reach the critical micelle concentration by the end of the induction period, the lipids self-assemble into reverse micelles, which enhance the rate of lipid oxidation. The presence of surface-active agents might stabilize these hydroperoxide-loaded micelles and inhibit branching reactions. α-Tocopherol itself may exert part of its antioxidant effect through this mechanism [13–15]. Phospholipids act as potent synergists for α-tocopherol in highly unsaturated fatty acids, such as fish oils [80, 81]. Phosphatidylethanolamine acted as a synergist in fish oil [82]. It was reported as a more active synergist of flavonoids in lard than phosphatidylcholine [83]. The phospholipids seem to exert their synergistic effect by acting as surfactants or emulsifiers stabilizing the hydroperoxide-loaded micelles and preventing the hydroperoxide decomposition and the initiation of new oxidation chains. This effect prolongs the induction period by preserving the primary antioxidants and extending their protective effect(s) [14, 41].

19.5 IMPORTANT ANTIOXIDANTS FOR FOOD STABILIZATION

Many synthetic phenolic compounds have been recognized as active antioxidants but only a few are used for food stabilization because of very strict safety regulations (Figure 19.5). Most of the approved phenolic antioxidants are substituted by more than one hydroxyl or methoxy group(s) in *ortho*- or *para*- position of the phenolic group, while *meta*-substituted compounds are nearly inactive. Synthetic phenolic antioxidants are mostly *para*-substituted due to lower toxicity while most natural phenolic compounds are *ortho*-substituted [84]. Among heterocyclic compounds containing nitrogen, only ethoxyquin (2, 6-dihydro-2, 2, 4-trimethylquinoline) was used but now exclusively in feeds [85]. Diludine (a substituted dihydropyridine derivative) is used for the stabilization of carotene and some pharmaceutical preparations but not in food in spite of its good activity in fats and oils [86]. Synthetic phenolic antioxidants are always substituted by alkyls (e.g., propyl, octyl, and dodecyl ester derivatives) to improve their solubility in fats and oils and to reduce their toxicity [87]. Mixtures of phenolic antioxidants often show synergistic activities, e.g., BHT and BHA [88]. In addition to their antioxidant activity, most phenolic substances possess antimicrobial activity in food [89]. It should be mentioned that synthetic α-tocopherol, D-ascorbic acid, and other antioxidants are considered as nature-identical compounds despite some differences. The mechanism of action of synthetic antioxidants is essentially the same as that of natural phenolic antioxidants. The only difference is that they are usually present in mixtures with related compounds of varying activities and/or with synergists.

By the end of the last century, there were concerns against using artificial chemicals, which led the food industry to turn to natural compounds for food stabilization [90, 91]. Almost all plants, microorganisms, fungi, and even animal tissues contain antioxidants of various types, which for various reasons (e.g., availability, food safety, and economical reasons) can be used as sources of antioxidants only in certain cases. The biosynthesis of antioxidants in plants was reviewed and

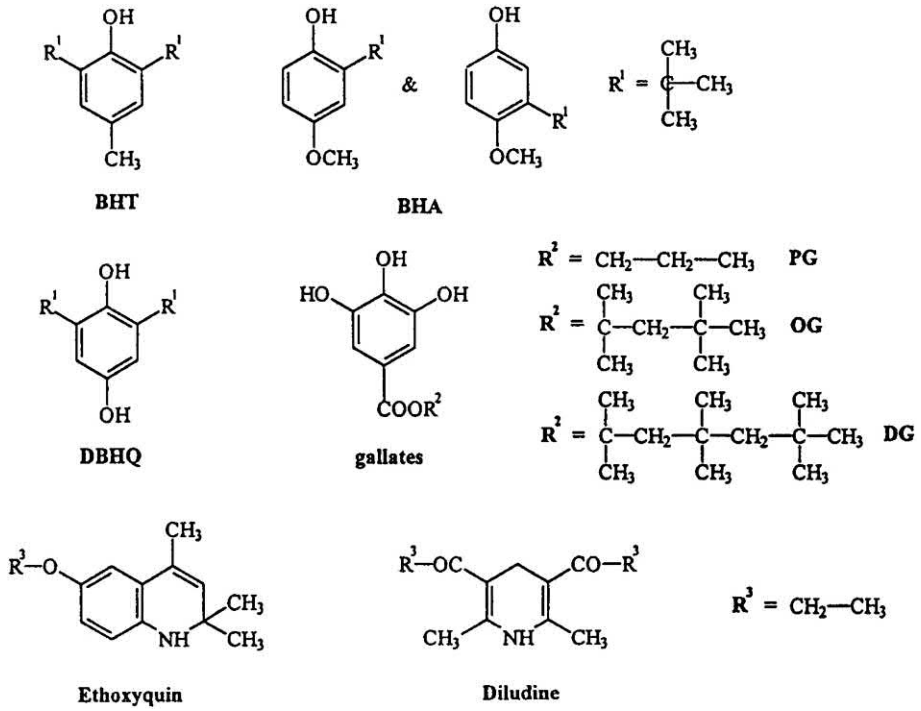

FIGURE 19.5 Chemical structures of the most important synthetic antioxidant used in food protection. BHT, di-tert-butylhydroxytoluene; BHA, tert-butylhydroxyanisole; DBHQ, di-tert-butylhydroquinone; PG, propyl gallate; OG, octyl gallate; DG, tert-dodecyl gallate.

discussed [92]. Research and development laboratories have shown great interest in exploring natural antioxidants and commercialized them as food protectants and life-saving phytochemicals. Since the use of food additives is subject to strict regulations, sometimes antioxidant extracts are added to foods as flavorings, coloring agents, or natural food components. In commercial preparations, natural antioxidants are mixtures of phenolic compounds and other compounds that would act as synergists, For instance, of 147 plants tested, 107 extracts showed appreciable antioxidant activity [93].

Although several natural antioxidant extracts can be used to protect foods against oxidation, their activities are generally low and they present unreproducible effects because they are mixtures of several compounds with different antioxidant properties, inactive impurities, or even prooxidants. When natural antioxidants are added to foods as unprocessed ingredients, the microstructure of the tissue and compartmentalization can play a decisive role in their antioxidant potential. The best method of application of natural antioxidants is to use natural food components (e.g., extracts of oilseeds, cereals, nuts, fruits, and vegetables) because they are regarded as safe, and no special approval for their application is necessary. Another possibility is to use natural food ingredients, such as spices. Natural compounds derived from nonfood materials, such as gingko leaves [94], should be tested for toxicity before application. A natural antioxidant, nordihydroguaiaretic acid (NDGA), extracted from the creosote bush grown in California, was originally used in food stabilization for a long time [95], especially in edible fats, but it is not permitted now because it has not passed more recently introduced safety tests. Because the biological variability of natural antioxidant extracts contributed to their unreliable activity, they were replaced by pure synthetic compounds that are cheaper, possess reproducible activities, and their safety have been tested and approved.

In higher plants, where phenolic compounds are very common, two series of compounds are of particular interest, derivatives of benzoic acid and cinnamic acid series [96]. The aromatic cycles substituted by two or three phenolic groups in the *ortho*- position are particularly important; some hydroxyl groups may be methoxylated. Gallic acid is a typical representative of the benzoic acid series, while caffeic acid is the most typical derivative of the cinnamic acid series. Catechins and flavones are more complicated compounds, where the antioxidant activity is located in a pyrocatechin or pyrogallol radical bound in the molecule.

Tocopherols and tocotrienols (Figure 19.6) are naturally present mostly in foods of plant origin. They are the most common natural antioxidants used in food preservation and they are present, at least in small amounts, in nearly all processed food materials. Tocopherols and tocotrienols are derivatives of chroman with a diterpenic (phytol) side chain; the active configuration is the phenolic group in the benzene cycle, located *para* to the oxygen atom bound in the adjacent dihydropyrone cycle [97]. There are four tocopherols, which differ in methyl substitution, the most important antioxidant of which is d-α-tocopherol, which has lower antioxidant activity than the other tocopherols but has the most vitamin E activity *in vivo*. The *in vivo* activity decreases in the order $\alpha > \beta > \gamma > \delta$ tocopherols [98], while the *in vitro* activity in bulk fats and oils decreases in the order $\delta > \gamma > \beta > \alpha$ tocopherols [99]. Their content in edible oils is particularly high

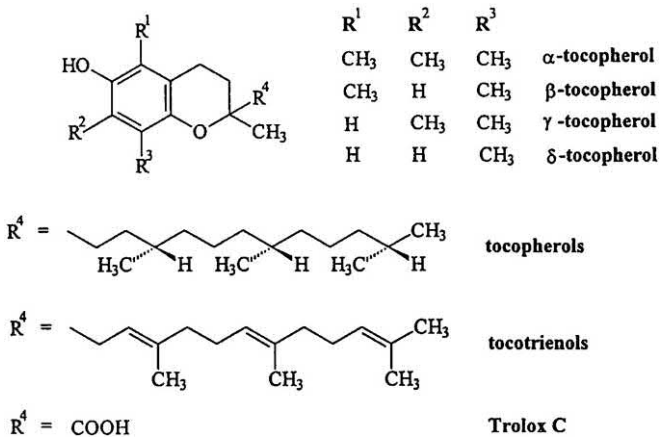

FIGURE 19.6 Chemical structures of tocopherols, tocotrienols, and Trolox.

(Table 19.4). Between 20% and 50% of the natural tocopherols are lost during edible oil refining (especially during the deodorization step), but they are often replaced by addition of α-tocopherol, tocopherol mixtures, or a deodorization concentrate (collected during the deodorization step of oil refining) into refined oils. Tocopherol acetate is added instead of free tocopherol because it is more resistant against oxidation during storage of edible oil. In foods of animal origin, such as butter or meat, tocopherols are present only in negligible amounts. In oilseeds, cereals, and other products, tocopherols are accompanied by dehydrotocopherols, tocomonoenols, tocodienols, and tocotrienols, which have one, two, and three double bonds in the side chain, respectively [100]. The last group is present in cereal flours and in palm fruits and palm oil. The related plastochromanol-8 has activity similar to α-tocopherol [101]. Tocopherols added to food products may be either synthetic products or natural concentrates, obtained most often from deodorization sludges from oil refining, from wheat or corn germs, or from other sources. Natural tocopherols have optical activity, unlike synthetic products. In foods of plant origin, additional tocopherols act as relatively weak antioxidants because vegetable oils already contain tocopherols in concentrations near the required optimum concentration. On the other hand, tocopherols are very active in vegetable oils that were stripped of their natural antioxidants [102]. Tocopherols are very active in foods of animal origin, as they contain nearly no natural antioxidants. A mixture of tocopherols, ascorbyl palmitate, and lecithin efficiently stabilized such a polyunsaturated material as fish oil [103]. By reaction with free radicals, tocopherols are converted into quinones, spirodimers, and various other compounds as well as in copolymers with oxidized lipids [97].

Hundreds of other natural antioxidants are known, some of them are shown in Figure 19.7. Several important oilseeds are sources of natural antioxidants other than tocopherols (Table 19.5) [104]. During the processing of oilseeds or oil-bearing fruits, antioxidants are partially extracted into crude oils. The best-known oxidation inhibitors are those present in olive fruits. Virgin olive oil, produced by pressing fruits under low temperature, contains several antioxidants derived from hydroxytrosol [105]. The antioxidants are present mostly as water soluble glycosides in the pericarp of the fruit and remain mainly in the residue after pressing (pomace). During ripening, storage, or pressing, glycosides may be hydrolyzed into the respective aglycones; for instance, oleoeuropein is present with its aglycone in virgin olive oils, but some antioxidants are partially reduced during pressing [106]. The content and properties of antioxidants depend on the degree of ripeness and the processing conditions [107]. Sesame seeds also contain phenol glycosides of lignan structure [108–111]. These compounds are only partially present in sesame oil where the most active compound is sesamol. The antioxidant activity is affected by roasting and steaming of seeds before extraction [112]. Sesame lignans, added in small concentration, were very active in ground pork and sausages [113]. Sunflower seed hulls are rich in polyphenols including melanins. Polyphenoloxidases oxidize polyphenols into quinones, which react with amino groups of proteins, imparting brown discoloration to seed meals. Therefore, extracted meals are frequently discolored by interactions of enzymatically oxidized polyphenols and protein. The seed contents of chlorogenic acid and related phenolic derivatives are only partially decomposed during processing. Peanuts contain flavonoids, tannins, and various other phenolic compounds concentrated in the hull [114, 115]. Cottonseeds contain gossypol, a polyphenolic compound with aldehydic groups possessing antioxidant properties. Because of the toxicity and contraceptive activity of gossypol, cottonseed meal cannot be used as feed

TABLE 19.4
Tocopherol Content in Vegetable Oils

Oil	α-Tocopherol (mg/kg)	β- + γ-Tocopherols (mg/kg)	δ-Tocopherol (mg/kg)	Total Tocopherols (mg/kg)
Soya bean	80–150	210–780	60–400	300–1400
Rapeseed	120–300	220–450	5–15	380–750
Sunflower	550–900	25–110	0–5	580–980
Corn germ	120–400	420–780	10–50	680–1250
Peanut	80–320	120–280	5–30	90–550
Olive	80–180	0–5	0	80–180
Cottonseed	550–700	200–280	0–5	800–980
Rice bran	500–650	230–340	0–5	750–950

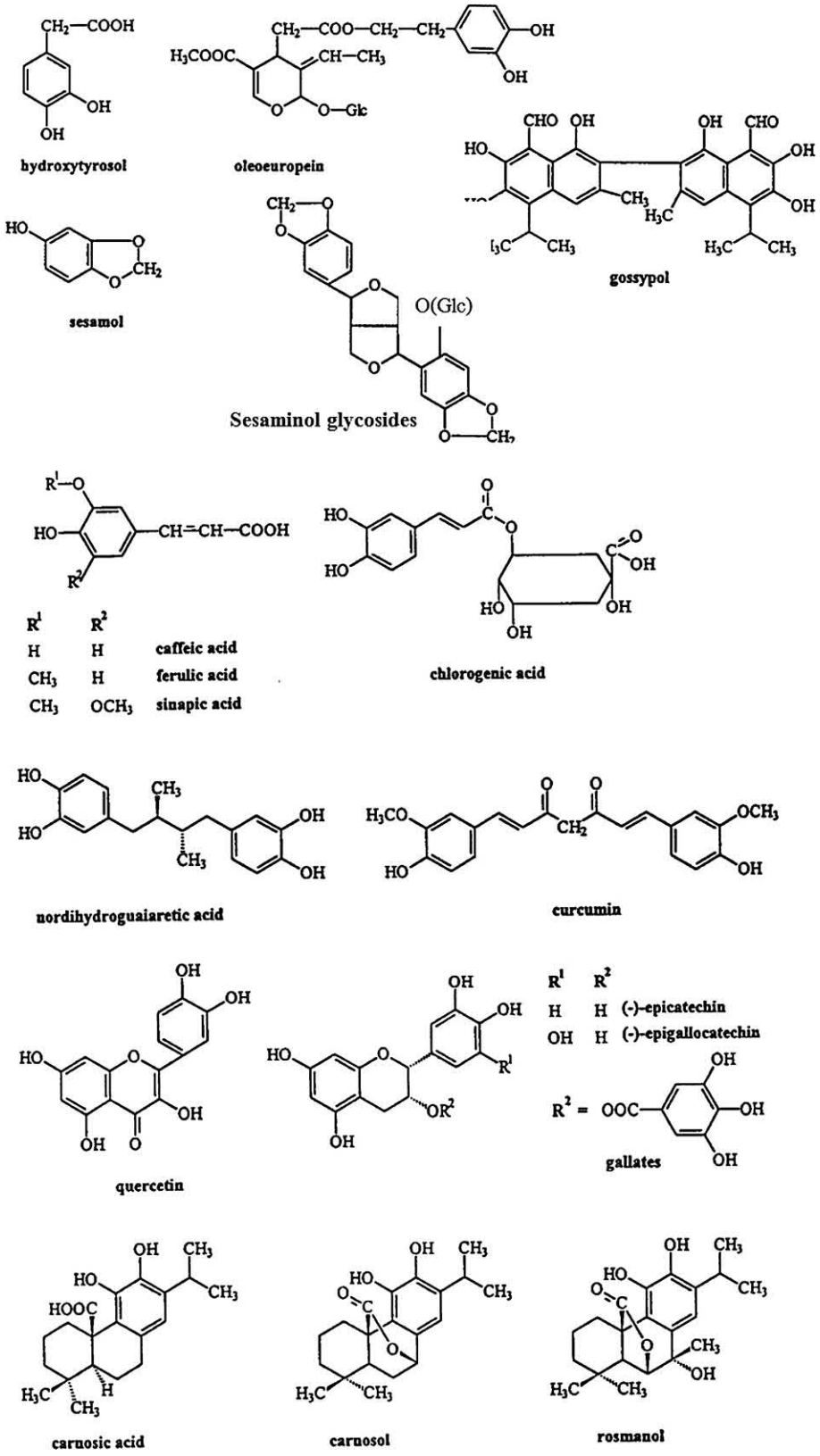

FIGURE 19.7 Chemical structures of selected natural antioxidants from seeds, herbs, and spices.

TABLE 19.5
Examples of Antioxidants from Selected Food Plants

Plant Sources	Latin Name	Main Antioxidants
Oilseeds and Cereal Grain		
Cottonseed	*Gossypium hirsutum*	Tocopherols, gossypol
Flaxseed	*Linum usitatissiumum*	Tocopherols, lignans
Olive fruit	*Olea europea*	Tocopherols, oleoeuropein aglycone
Palm fruit	*Elaeis guineensis*	Tocopherols and tocotrienols
Sesame seed	*Sesamum indicum*	Tocopherols, sesamol, sesaminol glucosides, sesamolin
Rapeseed	*Brassica napus*	Tocopherols, sinapic acid
Rice bran	*Oryza sativa*	Tocopherols, oryzanol
Fruits and Vegetables		
Betel	*Areca catechu*	Oleoresins, eugenol, hydroxychavicol
Carrots	*Daucus carota*	Carotenoids, flavonoids
Citrus	*Citrus* spp.	Flavonoids, carotenoids
Garlic	*Allium sativum*	Sulfides, disulfides
Green pepper	*Capsicum annuum*	Flavonoids
Green olives	*Olea europaea*	Phenolics
Legume	*Leguminosae*	Flavonoids
Mustard	*Sinapis alba*	Phenolics, isothiocyanates
Onion	*Allium cepa*	Sulfur compounds
Persimmon	*Diospyros kaki*	Procyanius, catechins
Pineapple	*Ananas comosus*	Flavanols
Plums	*Prumus spp.*	Phenolics
Red grapes	*Vitis vinifera*	Anthocyanins, co-pigments
Herbs and Spices		
Rosemary	*Rosmarinus officinalis*	Carnosic acid, carnosol
Sage	*Salvia officinalis*	Carnosic acid
Tanshen	*Salvia miltiorrhiza*	Carnosol
Thyme	*Thymus vulgaris*	Thymol, quinones
Savory	*Satureia hortensis*	Flavonoids
Clove	*Eugenia caryophyllata*	Eugenol, gallates
Black pepper	*Piper nigrum*	Ferulic acid
Ginger	*Zingiber officinalis*	Cassumarin, ginerol
Juniper	*Juniperus communis*	Phenolics, resins
Oregano	*Origanum vulgare*	*o*-Substitute phenolic acids
Fennel	*Foeniculum vulgare*	Dihydrocoumarins
Curcuma	*Curcuma longa*	Curcumin
Spearmint	*Mentha piperita*	Flavonoids
Lavender	*Levandula angustifolia*	Flavonoids
Hop	*Humulus lupulus*	Flavonoids, anthocyanins
Allspice	*Pimenta officinalis*	Flavonoids
Ginseng	*Panax ginseng*	Phenolic acids

in large amounts. Glandless, gossypol-free modern cultivars (glandless cottonseed) were developed. Gossypol-free cottonseed cultivars still contain flavonoid antioxidants such as quercetin and tocopherols [114, 116]. Soybeans also contain phenolic compounds with antioxidant activity, mostly flavones and isoflavones [117], which may stabilize lipids not only in beans but also in soy products, such as tofu or tempeh [118]. Isoflavones possess a hormone-like activity, which should be accounted for when evaluating the possible applications as inhibitors. Rapeseed (*Brassica napus*) and the related turnip rapeseed (*B. rapa*) are relatively rich in phenolic compounds, among which sinapic acid prevails and acts as a moderate antioxidant in oils rich in polyunsaturated fatty acids [117]. Rapeseed contains tannins as well as sinapic acid, which is mostly bound to choline, forming sinapine, which is nearly inactive as an antioxidant in oils. Evening primrose seed contains high levels of phenolics, mainly derivatives of protocatechuic and gentisic acids [119, 120]. Flaxseed also contains several lignans and flavonoids active as antioxidants [121].

Cereal gains, the most important components of the human diet, contain several types of phenolic compounds, especially in bran. Oat is considered relatively efficient from this standpoint [122] and oat extracts were the first natural antioxidants studied in detail before World War II. Extracts were patented and used in oils and other foods. Phenolic compounds present in oat seed are partially bound in lipids, and therefore are liposoluble. Rice bran is used for the production of oil; therefore, it is collected and extracted with solvents. During the process, some phenolic compounds, such as oryzanols [123], pass into crude rice oil. Rice oil is particularly rich in antioxidants (including tocopherols) and low in polyunsaturated fatty acids, therefore, it is very stable during storage like olive oil. Maillard products formed during the baking or roasting of food originate from sugars and amino acids; they have a stabilizing effect on lipids in foods, especially in baked products. The content of phenolic substances in grain legumes is mostly low; nevertheless, these might stabilize foods if added in substantial amounts as an ingredient. Phenolic substances (mainly flavanols) are concentrated in legume hulls [124]. These phenolic derivatives may be insoluble (tannins or lignins), but some derivatives are partially soluble in oil. This is why they sometimes become efficient in stabilizing the lipid fraction. Antioxidants from peas were isolated and their antioxidant activities reported [125]. Polar bean extracts showed high activities in lipid emulsions [126]. Lentil seeds are also a rich source of antioxidants, mainly phenolic acids [127].

The most important group of compounds active as antioxidants in fruits and vegetables consists of various flavones and related compounds [128]. Some substances belonging to this group act as cofactors of vitamin C protecting it from destruction by free radicals and increasing its vitamin activity. Ascorbic acid present in fruits increased the antioxidant activity of phenolic components in the linoleic acid–carotene system, e.g., in extracts from acerola fruits [129]. Grapes contain both lipophilic and hydrophilic antioxidants; their concentration is substantially higher in red wine than in white wine [130]. In red wine and deep-colored fruit juices, various anthocyanins are present [131], which are important antioxidants active in the aqueous phase. Red wine has been recommended as a source of antioxidants, such as resveratrol, to protect humans against the development of cardiovascular diseases, but several other factors may be involved so that the antioxidant activity should be confirmed by further experiments. In addition to the above compounds, various terpenic derivatives could act as potent inhibitors of lipid oxidation. The effect of terpenes will be discussed in the next section,

as terpenes are more important in spices. In the category of fruits, the substances investigated in most detail are those isolated from citrus fruits. Their activity is higher in oil-in-water emulsions than in oils because of the hydrophilic character of flavone glycosides and related compounds.

Ethanolic or aqueous extracts (which contain much more hydrophilic substances than hexane or ether extracts) seem to impart higher activities to water-in-oil emulsions. Their solubility also plays a role, therefore, they are more soluble in polar food systems, while hexane and ethyl acetate extracts are more soluble in fats and oils. Various nuts (e.g., macadamia nuts) contain antioxidants [128]; these substances are extracted into the respective oils by pressing. In peanuts and in almonds [132], phenolic antioxidants are concentrated in brown skins. Inhibitors of oxidation have been detected in extracts from hop [133]. Most among them are various pyrocatechol derivatives. Onion and garlic contain efficient inhibitors; because of their pungent flavor, they are suitable only for meat products, snacks, or cheeses [134]. Potent antioxidants can be obtained from various nonfood plant products, such as gingko leaves, but the substances from such sources (i.e., not used for food purposes) should be tested for safety before application.

Herbs are mostly leaves or stems from various plants used for the preparation of infusions, extracts, or soups. Many species of this group of food materials are active [92, 93, 135], mainly because of their content of phenolic compounds. The most important representatives of this group of substances are leaves from tea bush [136]. Green, oolong, and black teas are produced, depending on the technology of leaves processing [137]. Green tea contains a high percentage (about 20%) of catechins and related compounds [138]. The mixture mainly consists of catechins, epicatechins, gallocatechins, and the respective gallates. Extracts from green tea are, therefore, very active; their activity is comparable to that of synthetic antioxidants. Extracts from leaves of fermented, black tea are less active because a substantial part of the catechins has been converted into less active tea pigments [137, 139]. During the fermentation of tea leaves, catechins are partially enzymatically oxidized into the respective quinones, which dimerize into tea pigments—theaflavins and thearubigins. Wastes leftover after the preparation of tea infusions or commercial tea products may be used for the preparation of extracts with antioxidant activity. The antioxidant activity in lard may be ranked in descending order as follows: epigallocatechin gallate > epigallocatechin > epicatechin gallate > epicatechin. Raw materials for the preparation of herbal teas are less efficient, but various herbs used in China, Japan, and other Far East countries contain efficient antioxidants too [140]. Algae contain several brominated 3, 4-dihydroxybenzene derivatives [141]. Seaweed hydrolyzates were found very active as antioxidants, mainly in various animal foods [142]. Spices are used for conditioning meals and bakery products. Several species possess antioxidant activity [143], and the wastes left after the distillation of essential oils from spices could be used as raw material for the extraction of antioxidants.

The most active substances are those spices applied to foods as leaves or extracts of plant leaves [144–162]. Extracts of rosemary leaves are the only commercially available antioxidant of this group, and sage contains several structurally related antioxidants such as carnosic acid, carnosol, or rosmarinic acid. Their activity was tested in various foods, and particularly the lipid soluble fraction was active [161], e.g., in restructured pork steak [161]. Other active spices include thyme, juniper, oregano, ginseng, ginger, nutmeg, etc. In most cases, it is sufficient to add spices to the food products before or after the preparation, but extracts may be added instead of spices [153]. The addition of pure substances (isolated from spices) is not recommended, as the application of pure compounds would be considered as addition of antioxidants, not flavorings, and would be subject to legal restrictions. Some spices alter the flavor of food products, but deodorized material (i.e., after removal of essential oil by steam distillation) could be used without affecting the flavor. Spices that have antioxidant activity often possess antibacterial activity too [143].

In summary, wide ranges of water-soluble and lipid-soluble antioxidants are provided by nature, but as mentioned earlier, they face challenges due to their biological variability and irreproducibility. Currently, there is new room for research for the selection of antioxidant combinations and standardization for specific foods after the new knowledge on the role of antioxidants and synergists in the inhibition of lipid oxidation via chemical (radical scavenging) as well as physical (micelle stabilization) effects. This knowledge opens a new era for research and development, which might allow a combination of optimized antioxidant protection, packaging, and storage of foods.

19.6 APPLICATION OF ANTIOXIDANTS IN FOODS

The inhibition of lipid oxidation by antioxidants depends not only on their structures but also on many other factors, such as the composition of the lipid fraction, the presence of other inhibitors or promoters of oxidation, certain nonlipid components, water, the food microstructure, temperature, and packaging. Therefore, literature data should be always verified by tests in the specific food and its processing or storage conditions. Several synthetic antioxidants have proven to be effective, but their use is restricted to a few compounds, which have passed very complicated and expensive tests to prove their safety [163–165]. Such tests include the generation tests and teratogenic and carcinogenic trials using at least six species of animals, among which at least three species should be vertebrates and at least one species nonrodent. Therefore, no new antioxidants (with a few exceptions) are being tested and permitted, and the addition of the antioxidants to food is usually restricted to 0.02% on fat basis. Permitted antioxidants are different in different countries and subject to change [166]; some examples of antioxidants commonly used in the stabilization of fats and oils are given in Table 19.6. For practical reasons, it is suitable to add mixtures of antioxidants, which usually have higher activities than a single compound and which guarantee that the limits for single compounds have not been exceeded. Many commercial preparations are mixtures of antioxidants, synergists, and emulsifiers, or other substances facilitating

TABLE 19.6
Antioxidants Commonly Used in the Stabilization of Edible Fats and Oils

Antioxidant	Abbreviation	Applications
Propyl gallate	PG	Fats and oils
Dodecyl gallate	DG	Fats, emulsions
tert-Butylhydroxytoluene	BHT	Fats and oils, foods
tert-Butylhydroxyanisole	BHA	Fats and oils, foods
Di-*tert*-butylhydroquinone	DBHQ	Vegetable and fish oils, frying oils
Ascorbyl palmitate	AP	Vegetable oils, synergists
Tocopherols	TOH	General use in fats, oils
Citric acid, esters	CA	Synergists, edible oils
Thiodipropionic acid	ThA	Synergist
Lecithin	Lec	Synergist
Carotenes	Car	Frying oils, edible oils, emulsified oils
Silicone oil	SO	Frying oils

the dissolution of the product in fats and oils. Some natural materials are generally regarded as safe (GRAS); their use is not restricted by legislation (e.g., tocopherols, phospholipids, amino acids, etc.). Some common food ingredients, such as spices, are also not subject to legal restrictions as they are considered as flavoring or coloring substances. For pure compounds isolated from natural materials or extracts from plant materials not used as food, it is necessary to prove that there are no risks associated with their use.

Today, tendencies exist to avoid the "loss of efficacy" of antioxidants by reducing their concentrations in foods and by combining primary phenolic antioxidants with synergists (usually with the GRAS status). The antioxidant effect required by the producer and customers could, thus, be obtained with the lower addition of a primary antioxidant. Many examples have been published, e.g., by Banias et al. [167]. Theoretically, it is possible to prepare stable foods without added antioxidants. The easiest way is to modify the recipe to include components rich in natural antioxidants and to exclude components rich in polyunsaturated lipids. Another method is to prevent the access of oxygen, e.g., by impermeable packaging, often combined with packaging in a vacuum or in an inert gas, such as nitrogen. Oxygen may be removed by addition of oxygen scavengers, such as a combination of D-glucose and glucose oxidase. Storage at very low temperatures may be recommended, too. Nevertheless, there is a danger that when water freezes, ice crystals are produced and the product becomes dry. Under these conditions, the protective layer of hydrated proteins is damaged and the lipid fraction leaks from the natural emulsions or liposomes, so that lipids become exposed to air oxygen. The rapid turnover of foods in computerized food store facilities effectively eliminates the necessity of longer storage with the addition of antioxidants.

The efficiency of added antioxidants in foods depends very much on the food composition, microstructure, and moisture content [168]. Generally, dry foods, such as dehydrated soups, dried milk [169], and dried meat are very sensitive to oxidation, as air oxygen has free access to the film of lipids on nonlipidic particles. It may enter through tiny channels left after removal of water from the original material. The food stabilization is then less effective, as the initiation rate of the autoxidation reaction is relatively high and antioxidants are soon decomposed on storage or heating. In water-containing foods, the lipid fraction is relatively stable, as it is usually protected by a layer of hydrated proteins or carbohydrates against the access of air oxygen. The addition of nonpolar antioxidants is effective, while polar antioxidants may lose their activity by passage into the aqueous phase, similarly as in fat emulsions. Proteins and many other components of foods have protective action and act as synergist of other inhibitors, enhancing the effect of antioxidants [170]. Amine groups present in proteins do react with lipid hydroperoxides so that the level of free radicals decreases, which contributes to longer protection of foods against oxidation. Chelating agents are often present in foodstuffs as natural components, but heavy metals are usually present as well, for instance, heme derivatives in animal products. Because of the complexity of foods, it is necessary to test any addition of antioxidants to stabilize the particular material and to optimize the mixture of inhibitors.

Since vegetable oils contain nearly the optimum concentrations of tocopherols necessary for their stabilization [170, 171], the addition of phenolic antioxidants usually shows limited efficiency, but the addition of synergists is helpful. Ascorbyl palmitate, phospholipids, or organic acids (phosphoric, citric, or other polyvalent acids) are useful as synergists in vegetable oils. Most unsaturated oils are treated with organic acids during processing (the stage of deodorization) so that some residual synergists are often present in refined oils. Oils containing higher levels of natural antioxidants, such as olive oil, sesame oil, rice oil are sufficiently stable on storage without additional stabilization. Tocopherol acetate is sometimes added to increase the vitamin E activity and not to prolong the shelf life. On the other hand, animal fats, such as pork lard, beef tallow or milk fat, contain only very low amounts of natural antioxidants, therefore, their stability against oxidation is low despite their relatively low degree of unsaturation. Fortunately, both synthetic and natural antioxidants are very active in the stabilization of animal fats. Most often, mixtures of antioxidants and synergists are used for stabilization [172]. Lipid-soluble antioxidants show good results, but polar antioxidants could be used, too. When such fats, stabilized with polar antioxidants, are used for the preparation of food, e.g., for cooking or baking, the antioxidant activity is partially lost by extraction of antioxidants into the aqueous phase. Nonpolar antioxidants do not lose their activity as they remain in the lipidic phase creating a good carry-through effect.

Fish oils, including the oils of anchovy (*Engraulis* spp.), capelin (*Mallotus* spp.), cod and cod liver (*Gadus* spp.), herring (*Cupea* spp.), horse mackerel (*Scomber* spp.), tuna (*Euthynnus* spp.), menhaden (*Brevoortia* spp.), salmon (*Salmo salar*, syn. *Oncorhynchus* spp.), rainbow trout (*Oncorhynchus mykiss*), and sardine (*Sardina* spp.), account for about 2–3% of

the world's edible oil production and are the richest sources of nutritional eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The relative amounts of EPA and DHA in fish oils vary from 5–20% and 3–26% of total fatty acids, respectively [173]. Being highly unsaturated, EPA and DHA are highly susceptible to lipid oxidation and flavor deterioration and require stabilization with antioxidants. Fish oils, nutritional fish, and marine oil capsules are supplemented with tocopherols and other antioxidants, and properly protected from oxygen [174]. The stabilization of fish oils requires a mixture of tocopherols and synergistic phospholipids [175].

Frying oils present a special case, as they are exposed to air oxygen at high temperatures (130–200°C) during frying. Phenolic antioxidants are practically inefficient in frying oils because they are rapidly exhausted by the high rate of initiation reactions and because they are distilled off with water vapor released from fried food. Frying oils are efficiently stabilized by the addition of minute amounts of silicone oils, particularly siloxanes, which form a thin layer on the interface between oil and air [176], protecting against the access of air oxygen. Frying oils with low polyunsaturated fatty acid content, such as palm oil, palm olein, low-linoleic/high-oleic sunflower oil, and high-oleic peanut oil, are usually sufficiently stable under frying conditions that they do not require additional stabilization [177]. Interestingly, because of the low oxygen pressure during frying, carotenes and other hydrocarbons were found efficient in frying oils containing tocopherols as they form a monomolecular film on the surface, protecting frying oil against access of oxygen [178].

The activity of antioxidants in emulsions is very different from that measured in bulk fats and oils [25]. Polar antioxidants, such as propyl gallate or flavonoids, are extracted into the aqueous phase and only a small amount remains in the oil phase. Nonpolar synthetic antioxidants remain in the oil phase and only a very small amount incorporates in the aqueous phase but often they form less efficient micelles [16, 19, 20, 26]. Both polar and nonpolar antioxidants are accumulated at the intermediary layer between the two phases, which explains the polar paradox, i.e. polar antioxidants are more active in a nonpolar medium, whereas non-polar antioxidants are more active in the polar medium [12]. This paradox was shown in the case of the more polar Trolox versus the less polar α-tocopherol on the one hand, and polar ascorbic acid versus the less polar ascorbyl palmitate on the other hand when tested in bulk oil and in emulsion [179]. Some antioxidants of semipolar nature may accumulate on the water–oil interphase and prevent the diffusion of oxygen into the fat phase. The activity depends on the type of emulsion. Oil-in-water (O/W) emulsions are better protected against oxidation than water-in-oil (W/O) emulsions, as oil droplets are isolated from the access of oxygen by the aqueous phase and the interfacial layer. In the presence of tocopherols, phospholipids are active ingredients in oil–aqueous emulsions used as food [180]. When rosemary extracts are applied as antioxidants, the activity of antioxidants present in the mixture depends on the type of stabilized material. Carnosic and rosmarinic acids are more active than carnosol in oils, but carnosic acid and carnosol are equally active in emulsions, more so than rosmarinic acid [181].

Air oxygen penetrates the food material from the outer atmosphere, therefore, it is useful to protect food surfaces. Meat or fish products are often protected by packaging materials, impregnated with antioxidants, or by application of suitable antioxidants on the surface of materials, even when they are not packed. Most often, foods are distributed packed; the packaging material is then of great importance [182]. If the material is permeable for oxygen, antioxidants may be added to the packaging to inhibit the diffusion of oxygen. These antioxidants may enter into the packed food [183], especially foods with high lipid content. Obviously, the only antioxidants that can be used in packaging are those that are allowed in foods, and they should be applied only in such amounts that the content in foods does not exceed the legal limits. Packaging materials may be protected against oxidation by adding antioxidants already in the factory, even when the producer of foods does not intend to stabilize food in this way. This case is the same as earlier, but eventual extraction of antioxidants into food should be accounted for. The degree of migration of antioxidants from packaging should be tested for the respective type of food product.

New technologies such as the encapsulation of unsaturated fatty acids and antioxidants for safe delivery, e.g., in infant formulas, are being developed. In this case, it is very important to eliminate surface free oil that would otherwise spread on a large surface of the microcapsules and oxidize causing rancidity in that part of the food [184]. The encapsulation efficacy needs to be improved, e.g., by the use of multilayer encapsulation and the inclusion of different synergists and encapsulation agents such as protein hydrolyzates, gums, and carbohydrate polymers [185]. The design of the macrocapsules and selection of different materials is an open door for future innovations, especially for the protection of highly unsaturated omega-3 fatty acids [27].

19.7 ANALYSIS OF LIPID OXIDATION AND ITS PROTECTION BY ANTIOXIDANTS

The analysis of lipid oxidation and antioxidant protection is not a trivial task and often a single method is not sufficient and several analytical methods targeting the oxidizing substrate, residual antioxidant(s), and different reaction products are needed to identify and quantify the degree of oxidation [186]. The analysis of lipid oxidation and antioxidants has at least two aims: (i) the description of the status quo of the product and (ii) the prediction of the oxidative stability of the product under certain compositional and environmental circumstances. The methods that can be used are many and variable ranging from traditional methods (such as peroxide and *para*-anisidine values) to more sophisticated chromatographic and spectroscopic methods. Sensory analysis is less frequently used mainly because of the large variability in panelist scores. Measurements of total antioxidant activity or total antioxidant capacity are frequently used in literature to describe the antioxidant potential of plant extracts, but these

methods can only be indicative of high reactivity in a reaction tube and not necessarily in a food system.

Many methods have been used for the prediction of antioxidant activity in foods by determining the time length of the induction period at a given temperature [187]. The relative increase of the induction period due to the addition of antioxidants is called the protection factor. Protection factors are affected by the type of substrate and antioxidant. They are often high for low-unsaturated substrates containing no natural antioxidants, such as pork lard (of the order of 20–70) but low in polyunsaturated substrates containing sufficient tocopherols or other natural antioxidants (of the order of 2–4). Yanishlieva and Marinova [55] argued that the evaluation of antioxidant activity through the determination of the length of the induction period is not sufficient and presented a more complex model including the oxidation rate during the induction period, which is increased with the concentration for some antioxidants. They classified antioxidants as strong and weak; the strong ones increase the rate of oxidation during the induction period and are subject to loss of efficacy, although they might result in longer induction periods.

The best method for the determination of the shelf-life stability of lipids and the antioxidant activity would be storage of the food under the specific conditions, but this procedure would be too long and very expensive. Thus, accelerated methods (using higher temperature or catalysts) are almost exclusively used [188]. The oldest and most precise method is the Schaal oven test where the sample is stored at 40°C, 50°C, or 60°C in the dark under free access of oxygen. Changes are monitored either by determining the peroxide value or increase of weight for several days or months. In the active oxygen method (AOM) or earlier Swift procedure [189], the sample is incubated at 97.7°C (boiling water bath temperature) or 110°C (a glycerol bath). These procedures were used for a long time for rapid determination of antioxidant activity. Gases leaving the apparatus during the test can be monitored by sensory analysis, or they can be collected in water and estimated through changes of the carbonyl content or conductivity of the water phase. The increase in conductivity is due to the presence of lower fatty acids, especially formic acid, which originate in later stages of oxidation by secondary reactions. The Rancimat (Metrohm AG, Herisau, Switzerland) and the Omnion Scientific Oxidative Stability Instrument (OSI; Agilent Technologies Inc., North Kingstown, Rhode Island) are based on the same principle but the procedures are automated [190]. These two methods have been used a greater deal than the AOM method due to their simplicity, availability, reproducibility, and ease. The Oxidograph records changes in the amount of oxygen absorbed by the sample under very similar conditions. The results of accelerated methods do not correspond exactly to the results obtained under actual storage conditions [191]. Frankel raised objections against methods using high temperatures and recommended use of tests at lower temperatures, i.e., in the range of 20–60°C [192].

The American Society of Testing Materials (ASTM) Oxipres and Oxygen Bomb are based on recording the changes in pressure during oxidation at high oxygen pressure but without aeration, which is closer to actual conditions of food preparation and storage. The temperature can also be controlled to shorten the induction period. Various methods operating at lower temperature have been developed; in these cases, the oxidation is catalyzed either by photosensitizers or by the addition of copper ions. Their results may not correlate to those obtained during storage in the dark or without the presence of metals. Another method is the oxidation of an emulsion containing linoleic acid and carotene; changes of coloration or the increases of ultraviolet absorption are used for monitoring the reaction course. The antioxidant activity to scavenge free radicals is sometimes used for screening the suitability of antioxidants; various sources of free radicals are used. The method is rapid and simple, not requiring any expensive equipment.

Azo initiators, A-N=N-A, have been employed to test the activity of antioxidants at a constant initiation rate at a given temperature.

$$\text{A-N=N-A} \rightarrow \left[\text{A.N=N.A}\right]_{\text{cage}} \rightarrow 2\text{A}^{\bullet} + \text{N}_2 \qquad (19.24)$$

For example, lipid-soluble (2,2'-azobis-(2,4-dimethylvaleronit rile), AMVN) and water soluble (2,2'-azobis-(2-amidino-p ropane)-dihydrochloride, AAPH) azo-initiators are available for this purpose and have been used in many studies aiming to evaluate antioxidant potency [193]. However, results obtained with this method are still artificial and cannot account for the discrete reactions involved in the initiation of lipid oxidation during the induction period. In general, the method used for the determination of antioxidant activity should operate under conditions very similar to those of real application.

The degree of oxidation and the end of the induction period may be measured in different ways, such as on the basis of oxygen absorption or decrease of oxygen pressure, weight increase [167], increase of peroxide value, determination of conjugated dienes, or increase of 2-thiobarbituric acid reactive substances. Lipid hydroperoxides, the primary reaction products, are commonly determined by titration or spectrophotometrically. The method most commonly used is the official AOCS method, Cd 8b-90 [194], where hydroperoxides are reacted with iodide ions to form iodine that is titrated with thiosulfate using starch as an indicator:

$$\text{ROOH} + 2\text{I}^- + \text{H}_2\text{O} \rightarrow \text{ROH} + 20\text{H}^- + \text{I}_2 \qquad (19.25)$$

$$2\text{S}_2\text{O}_3^{2-} + \text{I}_2 \rightarrow \text{S}_4\text{O}_6^{2-} + 2\text{I}^- \qquad (19.26)$$

Alternatively, the combination blue complex product of iodine and starch can be measured spectrophotometrically at 550 nm [195]. The peroxide value (PV) is usually expressed as the milliequivalents of peroxides per kilogram of lipids but can also be divided by 2 and expressed as millimoles of active oxygen per kilogram lipids (SI units). Another method that is widely used to measure PV is the thiocyanate method based on the oxidation of ferrous salts to ferric salts by the hydroperoxides under acidic conditions and formation of a red complex

that can be measured by absorbance at 505 nm. The concentration of hydroperoxides with conjugated diene chromophore (CD) is sometimes estimated from the absorbance at 234 nm (molar absorptivity of E ≈ 28,000 $M^{-1}cm^{-1}$ in methanol). Other methods for the determination of PV exist but are used less frequently [196].

Because the PV has no simple relation to the actual rancidity due to the unstability, odorlessness, and tastelessness of hydroperoxides [197], it usually complemented by the *p*-anisidine value (AV), which measures the browning reactions of the respective aromatic amines with the aldehydes or ketones produced by the degradation of hydroperoxides. The resultant color intensity depends not only on the concentration of carbonyl groups, but also on the degree of unsaturation as well as on other factors [198]. The combination of PV and AV is referred to as the TOTOX value, which equals 2 PV + AV [196]. The 2-thiobarbituric acid value (TBA), determined by the spectrophotometric absorption of the condensation products of this reagent with malonaldehyde at 530 nm, is an additional measure of the oxidation of fatty acids containing three or more double bonds. If the condensation products are measured at 450 nm, it correlates better with total aldehydic oxidation products and rancidity than the substances reactive with formation of red products [199]. These spectrophotometric methods are easy, rapid, and cheap; however, their specificity is questionable. Several other spectrophotometric methods were reported in the literature, mostly based on a reaction with aldehydes, e.g., the conversion of aldehydes into 2,4-dinitrophenylhydrazones and the determination of color intensity.

Gas chromatography (GC) can be used for the analysis of unreacted polyunsaturated fatty acids as well as volatile compounds, dynamic headspace analysis, or using other analytical techniques [200]. The contents of propane, hexane, hexanal, 2,4-decadienals, and other products are correlated with the degree of rancidity. For the evaluation of the rancidity degree, the GC determination of volatile oxidation products, such as pentane, hexanal, or 2,4-decadienals, which are responsible for off-flavors, is the common method in use. Solid-phase microextraction (SPME), a solvent-free extraction technique, can be used for the sampling of volatile lipid oxidation products, but the method needs optimization and validation with respect to the extractability of the different compounds and the interpretation of the results [201]. The combination of SPME, GC, and mass spectrometry (MS), and the application of statistical techniques in data analysis improves the separation and allows a better selectivity and high precision [202].

High-performance liquid chromatography (HPLC) has limited applications in the determination of lipid oxidation products. However, it can be used for the analysis of lipid hydroperoxides, lipid hydroxycompounds, and ketodienes [53], especially when coupled with mass detectors. The best method for the determination of lipid oxidation products based on molecular weight is high-performance size-exclusion chromatography (HPSEC). This method has been applied for the analysis of lipid oxidation monomers, dimers, and oligomers e.g., in emulsion, dried-microencapsulated oils, frying oils, etc. [203].

Other methods that have been used in the analysis of lipid oxidation include nuclear magnetic resonance (NMR), electron-spin resonance (ESR), differential scanning calorimetry (DSC), infrared spectroscopy (IR), and chemiluminescence [204–207]. Oxidomics, i.e., monitoring of a number of oxidative changes in lipids simultaneously and evaluation of the results by chemometrics, have recently been applied to olive oil [208]. Application of these techniques is advantageous since it will highlight several changes by the same analysis and allows hypothesis-generation and broader conclusions than those that can be reached by the imprecise PV analysis [209]. Similarly, psychometric sensory analysis and related methods should be fundamental for their analysis of rancidity since it is a phenomenon of perception [197]. The degree of rancidity is the final goal of analysis, but usually sensory profile methods are used to estimate all flavor descriptors responsible for the final perception of rancidity. Even with expert panels of assessors, the analysis should be repeated 10–20 times to obtain reliable results. Electronic noses can provide an alternative and bridge between sensory evaluation and conventional chemical analysis of volatile lipid oxidation products in foods [210].

19.8 ANALYSIS OF ANTIOXIDANT POTENTIAL

In the analysis of the ability of natural antioxidant extracts to provide stability for food lipids, the two aspects are the determination of the content of antioxidants and their activity. The analysis of antioxidant content consists of two subsequent operation stages [165]: (i) isolation of antioxidants from the substrate and the purification of the extract, and (ii) quantification of antioxidants in the extract. Two difficulties are faced by the analyst: (i) how to achieve quantitative yield during the extraction and purification of extracts, and (ii) how to prevent the oxidation of antioxidants during the operation. Usually, antioxidants, being semipolar substances, are extracted using semipolar solvents, and the removal of solvents is performed under nitrogen. The extract may be purified by solid phase extraction on specific cartridges or using traditional column chromatography. Sometimes it is possible to use a precolumn for the subsequent chromatographic separation. Antioxidants naturally present or added to foods as natural components or flavorings may be partially converted into degradation products by reaction with oxygen or free radicals. Therefore, the content found by the analysis needs not exactly correspond to the amount originally present, similarly as in case of synthetic antioxidants. Several methods have been standardized by IUPAC [189] and/or AOCS [194]. Antioxidants are subject to changes during food storage or heating [54] so that it would be correct to isolate and determine not only the original antioxidant but also potential degradation products. Only in such a way, it would be possible to find whether the original content of antioxidants has not exceeded the legal limit.

Methods used for the determination of antioxidant content include spectrophotometric, chromatographic, and electrochemical methods (Table 19.7). Spectrophotometric methods are very simple and do not require expensive equipment but

TABLE 19.7
Methods for Analysis of Antioxidants

Method	Examples	Applications
Spectrophotometric	Redox titration with dichloro-phenolindophenol	Ascorbyl palmitate, propyl gallate, tocopherols
Chromatographic	Emmerie-Engel, Folin-Ciocalteu	Pyrocatechols, BHA, BHT
Electrochemical	GC after derivatization, reverse-phase HPLC, TLC, polarography, voltammetry	All antioxidants, gallates, ascorbic acid, tocopherols

suffer from several disadvantages including lack of specificity and meaning (see later). Antioxidant contents are better determined by HPLC using diode-array, fluorescence, electrochemical, or mass detectors [189]. Antioxidants are best determined with use of reverse-phase chromatography on octadecylated silica gel with ultraviolet detection. For the determination of very polar substances, such as propyl gallate or ascorbic acid, the direct phase would be acceptable. It can also be determined by gas chromatography [194] but after derivatization (silylation of phenolic hydroxyls). Thin-layer chromatography (TLC) was often used before the development of HPLC, and several standard methods based on TLC are still available [211]. TLC might be used with sophisticated equipment [212] such as high-performance TLC, or TLC and flame ionization detection (TLC-FID). Electrochemical methods are based on the determination of the amount of electric energy necessary to oxidize the substrate. The determination of all the three tocopherols, using pulsed voltammetry, is a typical example [189]. It is possible to combine HPLC with an electrochemical detector, e.g., CoulArray, to provide a very sensitive and selective method for sensitive phenolic profiling and quantification [213]. It is indeed possible to analyze phenolic compounds by liquid chromatography–mass spectrometry (LC-MS) of plant extracts, but the separation is complicated by the different forms in which each compound might exist, e.g., glycosylation or esterification forms [214]. Alternatively, plant extracts may be hydrolyzed before HPLC, but hydrolysis leads to considerable losses [215]. Phenolic compounds may also be analyzed by capillary electrophoresis with different detectors including mass detectors [216, 217].

The quantitative analysis of antioxidant extracts is necessary for their standardization and the handling of biological variabilities between cultivars, location, and other environmental variables. When the structures and quantities of component antioxidants are known, their antioxidant activity and stabilization of foods can be measured and related to the composition. For individual phenolic compounds, structure–activity relationships can be estimated based on their structures (hydrogen-donation ability and hydrophobic-lipophilic balance) and concentrations [26, 27]. Theoretical hydrogen-donation ability can be computed, e.g., by *ab initio* and electron functional-density approaches [218, 219], but the environmental effect of the food matrix significantly alters these theoretical values. Thus, new computational methods

TABLE 19.8
Selected Methods Commonly Used in the Determination of Total Antioxidant Activity/ Capacity of Plant Extracts

Method	Principle of the Assay	Detection of Change
Folin-Ciocalteu (Total Phenolic Content)	The antioxidant reduces molybdophospho-tungstic heteropolyacid, Mo(VI) (yellow) + e- (from AH) to give Mo(V) (blue). The assay is performed at alkaline conditions (pH = 10).	The presence of antioxidants decreases the absorbance of Mo(VI) at 765 nm.
DPPH (Diphenyl-Picryl-Hydrazyl Radical Assay)	The diphenylpicrylhydrzyl radical (DPPH), which is pink in color, is reduced by antioxidants to yellow color. The assay is performed at neutral conditions (pH = 7).	The presence of antioxidants decreases the absorbance, of DPPH at 515 nm
ORAC (Oxygen Radical Absorbance Capacity)	Radicals generated by AAPH react with β-phycoerythrin (β-PE) as a fluorescence probe and the reaction is inhibited by antioxidants. Fluorescence of β-PE is measured for 35 min after the addition of (2,2'-azobis(2-amidinopropane) -dihydrochloride, AAPH). The assay is performed at slightly alkaline conditions (pH = 7.4).	The presence of antioxidants restores the fluorescence of β-PE (Ex. 485 & Em. 520 nm).
ABTS (Trolox Equivalent Antioxidant Capacity)	A bluish-green radical cation ($ABTS^{\bullet+}$) is formed by the reaction of ABTS (2,2'-azinobis(3-ethylbenzthiazoline-6-s ulphonic acid) with sodium/potassium persulfate. Antioxidants reduce $ABTS^{\bullet+}$ back to ABTS. The assay is performed at slightly alkaline conditions (pH = 7.4).	The presence of antioxidants reduces the intensity of the initial blue color at 734 nm.
FRAP (ferric ion reducing antioxidant power)	Antioxidants reduce ferric tripyridyl triazine (Fe(III)-TPTZ) to ferrous form having intense blue color. The assay is performed at acidic conditions (pH = 3.6).	The presence of antioxidants inhibits the development of blue color at 595 nm.

and algorithms taking into account the nature of the food are needed for optimum utilization of antioxidant mixtures.

During the last two decades, a large number of methods have been used to determine the total antioxidant activity of plant extracts by reacting them with an oxidizing agent and/or a redox indicator and recording the change by spectroscopy [220–223]. These methods (Table 19.8 on previous page) measure the hydrogen donation ability of the phenolic compounds mixtures, which is the product of antioxidant activity and concentration. The main problem is the method and solvent used for the extraction of phenolic compounds that cover a wide range of polarity from glycosylated to esterified compounds. These methods are not sufficiently selective and their performance is relevant neither to the lipid oxidation mechanism nor to the effects of the food matrix. The reactions are usually performed in ethanol or methanol, intermediate polarity solvents that will eliminate the effects of micellization. These methods may be used to compare samples of the same plant for variation in the quality and antiradical performance of their phenolic compounds.

19.9 FUTURE TRENDS

Since foods are complex and variable systems ranging from simple oil-in-water and water-in-oil to multistructure–multiphase systems, the physical structure of the food significantly affects the rate of the chemical interactions between unsaturated lipids, oxidants, and antioxidants. The concepts of the polar paradox, the interfacial phenomena, and the role of micelles [12–27] are now ready to be refined and applied to different foods. Kinetic modeling of the reactions for individual foods, based not only on peroxide values, can be performed using sophisticated approaches such as oxidomics. In the near future, a better understanding of the antioxidant structure–activity relationship is expected, which will allow the design of more efficient antioxidant protection techniques and designs.

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20 pH in Food Preservation

Mohammad Shafiur Rahman and Md Ramim Tanver Rahman

CONTENTS

20.1 EFFECTS OF PH ON MICROORGANISMS AND ENZYMES

20.1.1 pH Values of Foods

The importance of pH on food stability and food preservation is well documented as well accepted by the scientific community. The term pH is the symbol for hydrogen ion [H^+] concentration. The hydrogen ion concentration of a food is a controlling factor in regulating many chemical, biochemical, and microbiological reactions. Hydrogen ion concentration is expressed in moles and pH is the negative log ion concentration. The pH scale ranges from 0 to 14. A neutral solution has a pH of 7.0. A lower scale reading indicates an acid solution, and a value above 7.0 indicates an alkaline solution. The pH scale is logarithmic rather than linear in character. Therefore, a pH of 3.0 is 10 times as acid as a pH of 4.0 [1].

The pH values of different food products are given in Tables 20.1 and 20.2. In general, fruits, soft drinks, vinegar, and wine possess low pH values at which most bacteria are unable to grow, and these products have good keeping qualities. Most meats, seafood, and raw milk have pH values greater than 5.6, which make them susceptible to bacterial spoilage and the possible growth of pathogens. Vegetables also have fairly high pH values and are more prone to bacterial spoilage [2]. Color, flavor, and texture of foods are also affected by pH [3].

The pH may vary considerably even within any given product. Some of the most important factors affecting the actual pH values of a product are [1] variety of cultivar, maturity, seasonal variations due to growing conditions, geographical areas, handling and holding practices prior to processing, and processing conditions. In the United States, the majority (93%, 355 out of 380) of beverages have a pH below 4.0 and 7% (25 of 380) shows a pH ≥4 [5].

20.1.2 Effects of pH on Microorganisms

20.1.2.1 pH Limits for Microbial Growth

Microorganisms require water, nutrients, and appropriate temperature and pH levels for growth. Table 20.3 lists the pH single factor limits for the growth of some important food spoilage and poisoning microorganisms. Minimum pH values for toxin production by *Clostridium botulinum* types A and B in canned foods are given in Table 20.4. This indicates that minimum pH levels are also dependent on the types of foods. The interaction of water activity and pH on toxin production by C*lostridium botulinum* type A and B in cooked vacuum-packed potatoes are given in Table 20.5. In general, heterotrophic bacteria tend to be least acid-tolerant among common food microorganisms [2]. A pH value of 4.5 is especially important because this is the pH below which *Clostridium botulinum* is widely regarded not to grow in foods. McClure et al. [6] reviewed the factors affecting growth and toxin production of *Clostridium botulinum*. They mentioned that grow and toxin production may occur at pH values below 4.6 if there is strict anaerobiosis, a high concentration of protein, and various acidulants used. Below about pH 4.2, most

TABLE 20.1
pH Values of Plant-Origin Foods

Food	pH	Reference
Apples	2.90–3.30	Booth and Kroll [2]
Apples	3.33–3.84	Murray [4]
Apple sauce	3.09–3.40	Murray [4]
Apricots	4.18–4.67	Murray [4]
Apricots (canned)	3.42–3.47	Murray [4]
Asparagus (cooked)	6.03–6.10	Murray [4]
Asparagus (canned)	5.20–5.32	Murray [4]
Bananas	5.00–5.29	Murray [4]
Bananas	4.50–4.70	Booth and Kroll [2]
Beans (cooked)	5.73–6.20	Murray [4]
Beans (canned)	4.62–4.72	Murray [4]
Beets (cooked)	5.23–5.90	Murray [4]
Beets (canned)	4.92–4.98	Murray [4]
Bread (white)	5.29–5.65	Murray [4]
Bread (wholemeal)	5.47–5.61	Murray [4]
Brussels sprouts	6.00–6.15	Murray [4]
Butter (peanut)	6.28	Murray [4]
Cabbage	5.40–6.00	Booth and Kroll [2]
Cabbage (green)	5.79–6.29	Murray [4]
Cabbage (cooked)	6.38–6.82	Murray [4]
Cantaloupe	6.17–7.13	Murray [4]
Carrots	5.88–6.00	Murray [4]
Carrots	4.90–6.00	Booth and Kroll [2]
Carrots (cooked)	5.58–5.88	Murray [4]
Carrots (canned)	5.18–5.22	Murray [4]
Cherries (red)	3.29–3.32	Murray [4]
Cherries (black, canned)	3.82–3.93	Murray [4]
Corn (canned)	5.90–6.44	Murray [4]
Coconut	5.90–6.52	Murray [4]
Cucumber	5.18–5.70	Murray [4]
Figs (canned)	4.92–5.00	Murray [4]
Grapes	2.80–3.80	Murray [4]
Grapefruit	3.22–3.70	Murray [4]
Grapefruit juice	3.00	Booth and Kroll [2]
Honey	3.70–3.78	Murray [4]
Lemon juice	1.98–2.40	Murray [4]
Lettuce	5.89–6.09	Murray [4]
Lime juice	2.00–2.25	Murray [4]
Melons	5.50–6.60	Murray [4]
Olives (green)	3.38–4.00	Murray [4]
Olives (ripe)	6.80	Murray [4]
Onions	5.32–5.85	Murray [4]
Oranges	3.30–4.30	Murray [4]
Orange juice	3.60–4.30	Booth and Kroll [2]
Peaches	3.30–4.05	Murray [4]
Peaches (canned)	3.70–3.82	Murray [4]
Pears (Bartlett)	3.49–4.08	Murray [4]
Pears (canned)	4.00–4.08	Murray [4]
Peas (cooked)	6.22–6.88	Murray [4]
Peas (canned)	5.71–6.00	Murray [4]
Pineapple	3.20–3.64	Murray [4]
Pineapple (canned)	3.39–3.50	Murray [4]
Plums (blue and Damson)	2.78–2.89	Murray [4]
Plums (red and yellow)	3.62–4.95	Murray [4]
Potatoes	5.40–5.90	Booth and Kroll [2]
Quince (stewed)	3.12–3.37	Murray [4]
Raspberries	3.62–3.95	Murray [4]
Rhubarb (stewed)	3.24–3.34	Murray [4]
Spaghetti (cooked)	5.97–6.40	Murray [4]
Spinach (cooked)	6.60–7.18	Murray [4]
Strawberries	3.30–3.35	Murray [4]
Tomatoes	3.99–4.75	Murray [4]
Tomatoes (canned)	4.10–4.28	Murray [4]
Vegetable soup (canned)	5.16	Murray [4]
Vinegar	3.12	Murray [4]
Watermelon	5.18–5.60	Murray [4]
Worcestershire sauce	3.63–3.67	Murray [4]
Yeast	5.65	Murray [4]

other food poisoning microorganisms are well controlled, but microorganisms such as lactic acid bacteria and many species of yeasts and molds grow at pH values well below this. Many weak lipophilic organic acids act synergistically with low pH to inhibit microbial growth. Thus, propionic, sorbic, and benzoic acids are the most useful food preservatives [7]. *Ichthyophonus hoferi* is an internal parasite of various fish species. Its growth was observed at all pH values 3 to 7, from 0°C to 25°C, and from 0% to 6% sodium chloride [8].

TABLE 20.2
pH Values of Animal-Origin Foods

Food	pH	Reference
Beef (broth)	6.14–6.20	Murray [4]
Beef (ground)	5.10–6.20	Booth and Kroll [2]
Beef (raw)	5.60	Murray [4]
Buttermilk	4.41–4.83	Murray [4]
Butter	6.10–6.40	Booth and Kroll [2]
Cheese (Camembert)	7.44	Murray [4]
Cheese (cheddar)	5.90	Murray [4]
Cheese (cottage)	4.75–5.02	Murray [4]
Cheese (Roquefort)	5.41–6.10	Murray [4]
Chicken	6.20–6.40	Booth and Kroll [2]
Cream	6.40–6.60	Murray [4]
Eggs (whole)	6.58	Murray [4]
Eggs (white)	7.96	Murray [4]
Eggs (yolk)	6.10	Murray [4]
Lobster	7.10–7.43	Murray [4]
Milk	6.30–6.50	Booth and Kroll [2]
Oysters	5.68–6.17	Murray [4]
Sardines	5.42–5.93	Murray [4]
Shrimp	6.80–7.00	Booth and Kroll [2]
Soda crackers	5.65–7.32	Murray [4]
Tuna (canned)	5.92–6.10	Murray [4]

TABLE 20.3
Low pH Limits or Growth Range for Microbial Growth

Microorganism	pH Range Value[a]	pH_{inside}	Reference
Acetobacterium species	2.8–4.3	4.0–6.0	Booth and Kroll [2]
Bacillus acidocaldarius	2.0–5.0	5.9–6.1	Booth and Kroll [2]
Bacillus cereus	4.9	—	Roberts [9], Walker [10]
Bacillus coagulans	3.7	—	Baird-Parker and Gould [11]
Bacteria	4–9	—	Booth and Kroll [2]
Campylobacter jejuni	5.3	—	Walker [10]
Clostridium botulinum	4.5	—	Baird-Parker and Gould [11]
Clostridium botulinum (proteolytic)	4.7	—	Roberts [9]
Clostridium botulinum (proteolytic)	4.6	—	Walker [10]
Clostridium botulinum (non-proteolytic)	4.7	—	Roberts [9]
Clostridium botulinum (non-proteolytic)	5.0	—	Walker [10]
Clostridium perfringens	4.5	—	Baird-Parker and Gould [11]
Clostridium perfringens	5.0	—	Roberts [9]
Clostridium thermoaceticum	5.0–8.0	5.7–7.3	Booth and Kroll [2]
Enterococcus faecalis	4.4–9.1	7.2–7.4	Booth and Kroll [2]
Escherichia coli	4.0	—	Baird-Parker and Gould [11]
Escherichia coli	4.4–8.7	7.5–8.2	Booth and Kroll [2]
Escherichia coli	4.4	—	Roberts [9]
Lactic acid bacteria	3.5	—	Baird-Parker and Gould [11]
Listeria monocytogenes	4.3	—	Roberts [9], Baird-Parker and Gould [11]
Lactobacillus species	3.0		Walker [10]
Molds	1.5–11	—	ASHRAE [12], Booth and Kroll [2]
Most *Bacillus* species	4.0	—	Baird-Parker and Gould [11]
Most yeasts and molds	<2.0–3.0	—	Baird-Parker and Gould [11]
Pseudomonas species	5.0	—	Walker [10]
Saccharomyces cerevisiae	2.35–8.6	6.0–7.3	Booth and Kroll [2]
Salmonella serovars	4.0	—	Baird-Parker and Gould [11]
Salmonella	3.8	—	Roberts [9]
Salmonella species	4.0	—	Walker [10]
Staphylococcus aureus (toxin)	4.5	—	Baird-Parker and Gould [11]
Staphylococcus aureus	4.0	—	Roberts [9]
Staphylococcus aureus (growth)	4.0	—	Roberts [9]
Vibrio parahaemolyticus	4.9		Roberts [9]
Yeasts	1.5–8.5		ASHRAE [2, 12]
Yersinia enterocolitica	4.4-4.6		Booth and Kroll [2], Roberts [9], Walker [10]

[a] Lowest or growth; values may vary according to particular food substrate and especially in the presence of organic acids.

The efficacy of acids depends to a large extent on their ability to equilibrate, in their undissociated forms, across the microbial cell membrane and, in doing so, interfere with the pH gradient that is normally maintained between the inside (cytoplasm) of the cell and the food matrix surrounding it [7, 15, 16]. In addition to the weak lipophilic acid, other preservatives most widely used in foods include esters of benzoic acid, which are effective at higher pH values than organic acids. The inorganic acids, such as sulfate and nitrite, are most effective at reduced pH values, like the organic acids. While these preservatives are employed at hundreds to thousands of parts per million (ppm) levels, the acids used principally as acidulants are often employed at percentage levels [7].

In many products, such as semidry sausages and cheeses, combinations of pH and water activity are used to preserve foods. The combined inhibitory effects of pH and water activity on survival of microorganisms are clearly additive [17]. In addition to pH, the type of acid is also a factor, which influences the extent of inhibition with water activity. Generally, citric and acetic acids tend to be more inhibitory in combination with water activity reduction than do hydrochloric or phosphoric acids [18]. The general effect of water activity and pH on growth of bacteria is shown in Figure 20.1 [17]. FDA's Good Manufacturing Practice Regulations (GMPR) governing the processing requirements and the classification of foods are shown in Figure 20.2. Low-acid foods packaged in hermetically sealed containers must achieve commercially

TABLE 20.4
Minimum pH Values for Toxin Production by *Clostridium botulinum* Types A and B in Canned Foods

Food	Minimum pH for Toxin Production
Prune pudding	5.44
Prune pudding	5.44
Pears	5.42
Pimientos	5.25
Pineapple rice pudding	4.94
Pork and beans	4.93
Zucchini	4.86
Vegetable juice	4.84

Source: Townsend et al. [13].

TABLE 20.5
Interaction of Water Activity and pH on Toxin Production by *Clostridium botulinum* Type A and B (Proteolytic) in Cooked Vacuum-Packed Potatoes

a_w	pH	Days to Toxin Detection
0.980	6.10	7
0.981	5.45	7
0.977	4.83	35
0.972	6.07	7
0.973	5.50	14
0.969	4.96	35
0.959	5.74	35
0.960	5.46	>35
0.964	4.95	>35

Source: Dodds [14].

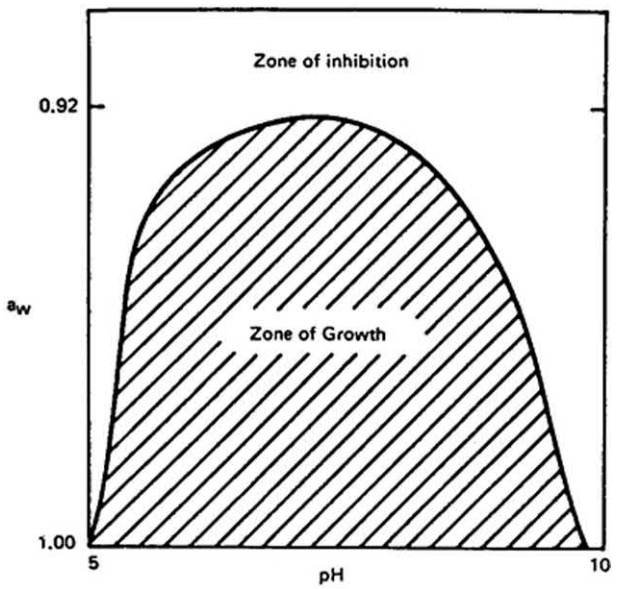

FIGURE 20.1 Interacting effects of pH and water activity on growth of bacteria [17].

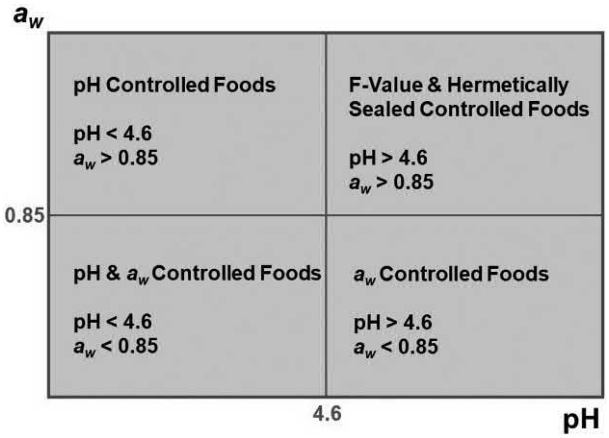

FIGURE 20.2 FDA Good Manufacturing Practice Regulations governing processing requirements and classification of foods. (From Johnston and Lin [19].)

sterile conditions either by retorting or a combined treatment of pasteurization and water activity (a_w), or a combined treatment of pasteurization and acidification [19]. Viruses, in contrast to bacteria, cannot imitate in food and water. Most of the foodborne viruses are more resistant to extreme pH than most vegetative bacteria [20]. The synergistic effect of pH, water activity, and temperature with different food processing technology can change the value of those parameters. Nonthermal processing technology, such as pulsed electric fields shows influence of pH, water activity, and temperature in killing of *Escherichia coli* and *Saccharomyces cerevisiae* [21].

20.1.2.2 Mode of Action of pH

Generally, bacteria can hardly maintain constant internal pH when they grow on a wide range of media of external pH. The H^+ ions destroy the amino–acid bond in nucleic acids, modify the cytoplasmic pH, and precipitate proteins; OH^- ions saponify the lipids in the enveloping membrane, leading to destruction of the superficial structure. A pH higher than 10.0 disorganizes the structure of the peptidoglycan and causes hydrolysis of the nucleotides of the virus genome. Similarly, the pH must exceed 12.0 to act on mycobacteria [22, 23]. Booth and Kroll [2] from Corlett and Brown [24] summarized three regimes of action:

- Strong acids, which lower the external pH but do not themselves permeate through the cell membrane. These acids may exert their influence by the denaturing effect of low pH on enzymes present on the cell surface and by lowering of the cytoplasmic pH due to increased proton permeability when the pH gradient is very large.
- Weak acids, which are lipophilic and permeate through the membrane. The primary effect of such acids is to lower cytoplasmic pH, and the undissociated acid may have specific effects on metabolism, which amplify the effects of the weak acid.
- Acid potentiated ions, such as carbonate, sulfate, and nitrate, which are more potent inhibitors at low pH.

As long as the internal pH of the microorganism remains constant, the effect of external pH on growth rate must be due to (i) inactivation of one or more essential enzyme activities, which are present on the outer layers of the cell (i.e., the outer membrane), the cell wall, the periplasm, and the inner membrane; and (ii) reduction of transport systems for essential ions and nutrients [2, 25].

At low external pH, the passive influx of protons under the influence of the proton motive force could be a major problem for cells attempting to regulate their cytoplasmic pH [26]. Most bacteria possess membrane-bound proton pumps, which exclude protons from the cytoplasm in order to generate a transmembrane electrochemical gradient of protons, the proton motive force. Those microorganisms, which are tolerant of very low external pH, acidophilic bacteria, and yeasts, have relatively low internal pH values. This may be a specific adaptation to the acidic environment [2]. The external pH has a significant effect on metabolism, often changing the pattern of enzyme synthesis and the nature of end products of metabolism. Booth and Kroll [2] concluded a reasonable generalization that cells produce acidic products when growing at alkaline external pH and neutral or basic products at external acidic pH.

A further counter to acidification of the cytoplasm is the buffering capacity provided by the acidic and basic side chains of the proteins and the phosphate groups of the nucleic acids. In general, buffering capacities in bacteria are approximately 400 nmoles H^+/pH unit/mg protein [27]. The buffering capacity is finite and can be overcome, for example, in the presence of high concentrations of a weak acid at low external pH [2].

In most microorganisms a cytoplasmic pH close to neutrality is essential for growth. For some microorganisms, recovery of cells from sublethal acid injury did not require new macromolecular synthesis, some cases of extensive protein denaturation occur in both bacterial and yeast cells, and protein synthesis is required for recovery [28–30]. DNA damage has also been suggested to occur in cells incubated at low pH [31]. Two generalizations can be made about pH homeostasis in microorganisms. First, the optimum cytoplasmic pH is species-dependent, i.e., acidophiles in the range 4.5–6.0, neutrophiles 7.5–8.0, and alkalophiles 8.4–4.9. Generally, yeasts and fungi exhibit similar values to the acidophilic bacteria [32]. Second, microorganisms exhibit different capacities to regulate their cytoplasmic pH and possibly have different tolerances of pH perturbation. For the purpose of pH homeostasis, it can be subdivided into fermentative and respiratory microorganisms [2].

20.1.2.3 Effects of pH on Heat Stability of Microorganisms

In the canning of foods, one of the most important factors affecting the sterilization times and temperatures is the actual pH value in the foods. The lower the pH values, the lower the degree of heat required for sterilization. The consumers' preference for acidic or nonacidic products also affects the selection of pH values. It is usually considered that a pH of 4.6 is the dividing line between acidic and nonacidic foods. Foods classified with respect to their pH values are shown in Table 20.6 [1]. Acidifying treatments for canned vegetables are also controlled by monitoring the pH values [33].

TABLE 20.6
Foods Classified According to Acidity

Group	Group Name	pH Range
I	Nonacid	7.0–5.3
II	Low or medium acid	5.3–4.6
III	Acid I	4.6–3.7
IV	Acid II	3.7–low

Source: Gould and Gould [1].

Bacterial spores are killed by heat more rapidly at low pH values than at pH values near neutrality [34–36]. Anderson and Friesen [34] showed that the rate of destruction of *Bacillus stearothermophilus* spores suspended in acetate buffer was only slightly more rapid at pH 4 than at pH 7, but below pH 4 the rate of death was much more rapid and appeared to be proportional to the proton concentration. At pH values of 7.0 and 6.0 spores of *Bacillus stearothermophilus* survived 60 min exposure unharmed at 100°C in the presence of lactic acid and sodium phosphate buffer, whereas at pH 4.3 and 3.0 they died with D_{100} (i.e., the decimal reduction times) values of 27 and 2.8 min, respectively. It was suggested that the enhanced death rate was due to toxic effects of undissociated lactic acid [37]. Watier et al. [38] measured the heat resistance of two strains of spoilage bacteria *Megasphaera cerevisiae* at temperatures 50°C to 60°C. The values of D_{50} were lower at pH 5.2 and 6.0, while at pH 4 the heat resistance was 4.2 times lower, thus at low pH the destruction rate was much higher. Koutsoumanis et al. [39] showed that exposure to a mildly acidic (pH 5.0 to 6.0) environment provided protection of the *Listeria monocytogenes* against acid.

20.1.2.4 Enhancement of the Effects of Preservatives

The efficacy of any preservative depends on the pH levels. Michener et al. [40] investigated 650 compounds for the ability to increase the susceptibility of spores to heat at pH 7. *Bacillus stearothermophilus* spores died much more rapidly at 100°C and mean pH 3.5 in the presence of lactic acid than in its absence. At pH 3.5, lactic acid (pK_a, 3.87) is approximately 70% undissociated and it can be suggested that the enhanced death rate was due to toxic effects of the undissociated acid [37].

20.1.3 pH Effects on Enzymes

Lipoxygenase catalyzed the oxidation of unsaturated fatty acids resulting in an off-flavor. Complete inactivation of lipoxygenase was irreversible when treated at pH 3.0 and below [41, 42] and inactivation of (i.e., activity) of urease was reduced to a commercially acceptable level at that pH. No effect on trypsin activity was observed up to pH 2. More than 70% protein dispersion ability was retained in the neutralized

full-fat soy flour after treatment with acid at about pH 3.0 [42].

Pectin esterase de-esterifies pectin, which leads to cloud loss in citrus juice. The thermal inactivation rates of pectinesterase in citrus juices increased at lower pH values [43]. The effect of pH on stability of thermolabile and thermostable pectinesterase was studied by Sun and Wicker [44]. Both isozymes showed stability over a wide pH range. Thermolabile pectinesterase was inactivated irreversibly at pH 2 and 12, whereas thermostable pectinesterase maintained almost the same activity as before pH treatment with slight inactivation at pH 12. Thus, the stability and conformation of thermostable pectinesterase were less likely to be changed by low pH treatment, and the conformational change was nearly reversible.

The enzyme lysozyme has antimicrobial potential to prevent or delay microbial growth in a variety of foods, such as fresh fruits and vegetables, tofu bean curds, meats, seafood, cheese, and wines. The relatively high thermal stability of lysozyme also makes it attractive for the use in pasteurized and heat-sterilized food products, possibly allowing reduced thermal processes, therefore, minimizing nutritional and sensory quality loss [45]. Makki and Durance [45] studied the stability of lysozyme in aqueous buffer solutions at selected temperatures (73–100°C), pH values (4.2–9.0) and sucrose (0%, 5%, 15%), and sodium chloride (0, 0.1, 1 M). Lysozyme was most stable at pH 5.2, and thermal stability decreased sharply as the pH increased to 9.0. At pH 7.2 and 9.0, sodium chloride had a clear stabilizing effect against heat inactivation of lysozyme. Sucrose stabilized lysozyme against heat inactivation at 75°C but not at 91°C. Loss of activity followed first-order kinetics, and a rate constant correlation was developed in the pH 5.2 to 7.2 range and temperatures between 73°C and 100°C as follows:

$$\ln k = \frac{32.90 - 1.62 \times 10^4}{T + 1.19\,(\text{pH})} \tag{20.1}$$

Ibrahim et al. [46] found that heat denaturation of lysozyme at increasing temperatures (80°C at pH 7.0 or over 90°C at pH 6.0) for 20 min resulted in progressive loss of enzyme activity while it greatly promotes bactericidal activity against gram-negative and -positive bacteria. They also observed that action is independent of catalytic function and kills bacteria through the membrane damage mechanism as found from electron microscopy.

20.2 EFFECTS OF PH ON FOOD COMPONENTS

20.2.1 Effects of pH on Gel Formation

The pH also affects many functional properties, such as color, flavor, and texture of foods, although the pH of a food is important for microbial growth. Acid fruit pulps form weak gels that collapse under their own weight [47, 48]. Texture formation of these acid pulps below pH 3.5 involves an important dilution of the pulp, thus pulp should be neutralized by addition of sodium hydroxide [49, 50].

Egg albumen is an important food ingredient because of its ability to incorporate other ingredients through the formation of a three-dimensional gel matrix. Gel properties are dependent upon many variables, including pH and ionic strength as well as the salts present. Savoie and Arntfield [51] studied the gelation of ovalbumin in the presence of salts containing Ca^{+2} and Mg^{+2} at various pH values. The impact of this binding on gel structure was dependent on pH and the technique used to evaluate structure. At pH 5, proteins tended to coagulate regardless of the type or amount of salt. At pH 7, the highest rigidity values from penetration measurements were obtained at salt levels of 0.005 M. At pH 9, the salt concentration for maximum rigidity varied with type of salt, while the storage moduli from dynamic rheology were highest at 0.01 M.

A significant improvement of functional properties, including foaming, emulsifying, and gelling properties of dried egg white (7.5% water) on the protein heating in a dry state at 80°C for several days [52]. Mine [52] found that heating of dried egg white proteins in the dry state at alkaline pH (under 9.5) was an effective method to obtain firm and elastic gels. The degree of unfolding of the proteins upon dry heating may play a crucial role in the gelling process of the proteins. The polymerization of the proteins was also enhanced by alkaline dry heating through the sulfhydryl–disulfide interchange. Alkaline dry heating resulted in a high molecular weight polymer of partially unfolded egg white proteins, which in turn contribute in the formation of low molecular weight and narrow molecular distribution of the aggregate.

The sinapic acid and thomasidioic acid bind to canola protein and affect protein functionality, especially gel formation upon heating of protein. Rubino et al. [53] studied the influences of sinapic and thomasidioic acid on the rheological characteristics of canola protein gels. At pH 4.5, there was binding between sinapic acid and the canola protein through electrostatic interactions, while at pH 7 and 8.5 there appeared to be a hydrophobic association between thomasidioic acid and canola protein. The presence of either compound resulted in deterioration of the characteristics of heat-induced gels for the canola protein. Sunflower protein gelation is strongly pH dependent. Sanchez and Burgos [54] found that gelation was only possible in the pH range 7–11 for sunflower protein, the storage modulus reached its maximum value at pH 8, the gels formed at pH 7 or above pH 9 were very weak, gelation time increased with pH and decreased with protein concentration, and the storage modulus at pH 8 increased exponentially with protein concentration.

20.2.2 Effects of pH on Proteins

The behavior of proteins is pH dependent and each protein has an isoelectric point (pI), where the contributions from positive and negative charges cancel out to give the molecule no net charge. At this pH, proteins tend to coagulate, and therefore it is to be expected that surface rheological parameters of proteins close to their isoelectric point are maximal [55, 56]. At the pI, charge-based contributions to repulsion are minimal, and steric stabilization is minimized since the proteins are

in their least expanded state [55]. The emulsion stability in some systems is greatest at the pI, such as gelatin [57], bovine serum albumin [58], pepsin [58], and soluble muscle protein [59]. This is probably due to the greater surface coverage at the pI with the compacted protein structures, and together with the tendency of the protein to coagulate at the pI gives cohesive films their enhanced stabilizing action [55]. Some proteins are shown to give less stable emulsions at the pI, such as low concentrations (0.004%) of either bovine serum albumin or lysozyme may be due to surface coverage, milk fat/whey protein, which are highly unstable at pH 4.5–5.0, close to isoelectric points [60]. Thus, Dalgleish [55] concluded that the stability of emulsions at the isoelectric point (i.e. pl) is dependent on protein concentration, the volume–surface area of the oil phase, and pH.

At other pH values, proteins may show a distinct dependence on pH [28]. Bovine serum albumin showed increasing emulsifying activity as the pH was increased between 4 and 9, and then decreased sharply as the protein changes conformation [61–63]. In the range of pH 3–8, β-lactoglobulin did not change its emulsifying capacity, although it passed conformational transitions [63]. Shimizu et al. [64] found a strong dependence of the emulsifying power with pH, increasing from pH 3 to 9. The hydrophobicities of the different whey proteins vary with pH, but all of the proteins behave similarly, i.e., their surfaces become less hydrophobic as the pH is increased [65]. Increasing the ionic strength diminishes the charged-based interactions between proteins and produced the same type of effects as changing the pH toward the isoelectric point [55].

Agboola and Dalgleish [66] studied the effects of pH and ethanol on the kinetics of destabilization of oil-in-water emulsions containing milk proteins. Under shear, emulsions containing caseinate were stable between pH 3 and 3.5 and at pH $\geq$ 5.3, while those formed with β-lactoglobulin were stable below pH 4 as well as at pH $\geq$5.6. The kinetics of pH-induced aggregation in emulsions could be explained by orthokinetic flocculation while ethanol-induced association in caseinate emulsions appeared to be a result of Ostwald ripening.

Caseins comprise approximately 80% of the total protein content in milk. Caseins are phosphoproteins precipitated from raw milk at pH 4.6 at 20°C. The α_{s1}-caseins contain more acidic amino acids than basic ones, with a negative net charge of 22 at pH 6.5 [67]. β-Lactoglobulin is remarkably acid-stable, resisting denaturation at pH 2.0. The protein assumes the shape of a prolate ellipsoid with an axial ratio of 2:1 and a hydration ratio of 35% to 40% [68]. It generally exists as a dimer resulting from the association of the monomer at the respective α-helical segments at the isoelectric pH of 5.2 and alkaline pH range. β-Lactoglobulin A at low temperatures and high concentrations, between pH 3.5 and 5.2, tends to form octamers as the predominant species. Below pH 3.5, β-lactoglobulin dissociates into monomers due to electrostatic repulsion between the subunits. Above pH 6.5, the dimers begin to dissociate. A transition in conformation occurs near pH 7.5, which is reversible and involves only a certain region of the molecule [69–71]. This reversible unfolding is followed by slow changes, which become increasingly irreversible with increasing pH [67]. Bovine α-lactalbumin is insoluble at the isoelectric range between pH 4 and 5. In contrast, goat α-lactalbumin forms a clear solution over a wide pH range. In the physiological state, it exists in the calcium-bound form. The calcium is tightly bound and is not removed by isoelectric precipitation and dialysis against phosphate buffer [72, 73].

20.2.3 Effects of pH on Vitamin Stability

The stability of vitamins depends on the pH of the medium and are summarized in Table 20.7 under acid/alkaline conditions. At higher water activity (>0.90), the rate of thiamin hydrochloride (vitamin B_1) degradation in a buffered solution of glycerol at 85–95°C is independent of water activity but highly dependent on pH [74]. Thiamin hydrochloride was less stable in solutions of univalent ions than in glycerol or divalent ions at the same water activity [75]. Riboflavin is also sensitive to pH. Nguyen and Hendrickx [76] studied the degradation of 5-$HCOH_4$ folate as a function of pH. They found some lability in acidic conditions and better stability in a broad zone between pH 5 and 8. Kinetic constants for the degradation of folate vitamins under various pH conditions are reported by Delchier et al. [77] when degradation of folate is first-order kinetics.

20.2.4 Effects of pH on Food Color

The colors of fruits, vegetables, and meats are associated with pigments (e.g., anthocyanins, chlorophyll, carotenoids, and myoglobin) and these pigments are influenced by pH. Wahyuningsih et al. [78] showed anthocyanin with a low pH or high pH has a significant effect on the food colorant.

TABLE 20.7
Stability of Vitamins under Acid/Alkaline Conditions

	Condition[a]		
Vitamin	**pH = 7**	**Acid Medium**	**Alkaline Medium**
Vitamin A	S	U	S
Vitamin D	S	—	U
Vitamin E	S	S	S
Vitamin K	S	U	U
Vitamin C	U	S	U
Vitamin B_1	U	S	U
Vitamin B_2	S	S	U
Vitamin B_6	S	S	S
Vitamin B_{12}	S	S	S
Niacin	S	S	S
Pantothenic acid	S	U	U
Biotin	S	S	S
Folic acid	U	U	S

Source: Murray [4].

[a] S: stable; U: unstable.

There is general understanding that the fundamental cause of green vegetable discoloration during processing is the transformation of chlorophylls to pheophytins by the impact of pH. The green color of vegetables swings to an olive green when warmed or set in acidic conditions. The amount of ferrihemochrome formation from myoglobin during cooking is affected by initial meat pH; mammalian muscle has a pH of around 7 and normal fresh meat has a pH ranging from 5.4 to 5.6 [3].

20.2.5 Effects of pH on Food Texture

Consumers' acceptability of food is influenced by texture and texture is affected by pH. Brandt et al. [79] observed firmed cauliflower, beans, potatoes, peas, and corn at pH 4 in the cooked state. In general, the vegetables are softest at pH 10; at pH 2, the firmness was only surpassed by the action of pH 4. Muscle pH has been associated with numerous other meat quality attributes including tenderness, water-holding capacity, cooking loss, and microbial stability (shelf life). Marination is widely used by consumers to improve meat tenderness and flavor. In this process pH plays a vital role in marinated meat. This improvement is due to the swelling of muscle fibers and/or connective tissue [3].

20.2.6 Effects of pH on Food Flavor

The acceptance of food by consumers is influenced by the flavor of that food. In Maillard reaction, flavoring compounds are formed and the flavor compound formation depends on the type of sugars and amino acids involved, reaction temperature, time, pH, and water content [80]. Several studies on Maillard reaction model systems have demonstrated that the pH of the reaction medium results changed in volatile and colored products. So the pH strongly determined the types of volatiles formed. Furthermore, flavors are very susceptible to change by the action of pH, moisture, acid, salt, and enzymes.

20.3 METHODS OF CONTROLLING PH IN FOODS

The pH of foods can be altered by (i) adding acidulants, such as acetic, citric, ascorbic, and lactic acid; and (ii) the action of microorganisms in many foods, such as cheese, yogurt, meat, and alcoholic beverages; in this method, growth of spoilage microflora is controlled by the production of lactic or acetic acids.

In fermentation, carbohydrates and other reduced substrates are incompletely oxidized in the absence of an external electron acceptor. In this process, all the electrons removed from a fermentable carbon source during its oxidation to release energy must be consumed by the reduction of a carbon metabolite resulting in the formation of a fermentation product [2]. The production of acid by fermentation plays a significant part in the preservation of food. In many dairy products, the production of lactic and acetic acids, and hydrogen peroxide may also be an important factor. With meat, it is considered that the reduction of pH and not the production of lactic acid is primarily responsible for the preserving action [2, 81, 82]. In dairy fermentations, flavor production is very important. The principal flavor components include acetaldehyde and diacetyl, which are by-products of the fermentation [2].

Fermentation processes have been applied to fish for many years and represent a low level and affordable technology for tropical developing countries [83]. Fagbenro and Jauncey [84] studied fermented tilapia stored for 180 days. Fagbenro [83] developed a preservation method for raw shrimp heads by fermenting with 5% (w/w) *Lactobacillus plantarum* as the inoculum at 30°C using 15% (w/w) cane molasses as a carbohydrate source. After incubation for 7 days, a desirable and stable pH <4.5 was attained in the anaerobic treatments, which lasted until 30 days after the start of fermentation. The addition of trona at 5% prior to fermentation restricted protein hydrolysis by inhibiting the activity of endogenous autolytic enzymes. The addition of onion extract at 5 ml/kg proved effective as an antioxidant, as the value of the thiobarbituric acid reactive substances remained low after 30 days of fermentation.

20.4 CONCLUSION

The pH of foods can be altered by adding acids and by adding beneficial microorganisms, such as lactic acid bacteria and fermentation. pH plays a critical role in food processing and preservation by affecting microbial, chemical, biochemical, and physical changes. A pH value of 4.5 is considered critical since *Clostridium botulinum* and most pathogens are unable to grow. In controlling microorganisms, weak acids are more effective than strong acids. In addition to pH, other factors such as water activity, temperature, and preservatives also affect the critical limits as well as the growth and death rate of bacteria. The pH also affects the gelling characteristics, protein, enzyme and vitamin stability, and production of the desired color, flavor, and texture. However, prediction of the stability of the combined effects of pH and other factors is a challenge.

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21 Nitrites in Food Preservation

Mohammad Shafiur Rahman

CONTENTS

21.1 NITRITES AS CHEMICALS

Preservatives are compounds used to delay or prevent the chemical and microbiological deterioration of foods. Nitrites and nitrates are used in many foods as preservatives and functional ingredients. Nitrites are critical components used to cure meat and are known to be multifunctional food additives. They are also potent antioxidants. Nitrites are white to pale yellow hygroscopic crystals. Sodium nitrite ($NaNO_2$) is markedly less hygroscopic than potassium nitrite (KNO_2). Nitrites are quite soluble in water and liquid ammonia but much less soluble in alcohol and other solvents. At room temperature, one part of water dissolves one part sodium nitrite or three parts potassium nitrite [1].

21.2 ANTIMICROBIAL EFFECTS

Sodium nitrite plays an important role in inhibiting the growth and toxin production of *Clostridium botulinum* in cured products [2]. Input concentrations in excess of 100 mg/kg are used to produce protection against microflora [3]. The review of Woods et al. [3] indicates a 200 mg/kg at pH 6.0 was capable of inhibiting strains of *Achromobacter*, *Aerobacter*, *Escherichia*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas* species. *Salmonella*, *Lactobacilli*, and *Clostridium perfringens* are more resistant when compared with other clostridia [3]. Times to first swell were 6.7, 29.8, 82.6, and 94.3 days when 0, 50, 100, and 156 mg/kg of sodium nitrite, respectively, were added to the perishable canned cured meat [4]. The primary effect of nitrite appeared in determining the length of the lag phase. Once swelling commenced, the rate at which the cans swelled was not significantly different at 50, 100, and 156 mg/kg of sodium nitrite. At 50 mg/kg nitrite, a 75% probability of toxicity was predicted at 3 months. Hauschild et al. [5] concluded that (i) the degree of safety from *C. botulinum* toxin production can differ by several orders of magnitude depending on the composition of formulation, and (ii) the large reductions in the concentrations of nitrite in the products could produce severe consequences.

21.2.1 Stage of Inhibition

Germination and outgrowth of bacterial spores include five sequential steps: (i) germination (i.e., becoming nonrefractile, stainable, and heat-sensitive), (ii) swelling of the germinated spore, (iii) emergence of new vegetative cell, (iv) elongation, and (v) cell division [6]. The inhibitory effect of nitrite

on bacterial spore formers is apparently due to inhibition of outgrowth and during cell division [7–9]. Duncan and Foster [6] identified points of inhibition in the outgrowth process of anaerobic spores. Up to 0.06% at pH 6.0 or between 0.8 to 1% at pH 7.0 nitrite allowed emergence and elongation of vegetative cells but blocked cell division. Elongated cells did not multiply, and eventually lysed by leaving the empty spore coats. With more than 0.06% nitrite at pH 6.0 or more than 0.8 to 1% at pH 7.0, the spores lost refractility and swelled, but vegetative cells did not emerge. Even as much as 4% nitrite failed to prevent germination (i.e., complete loss of refractility) and swelling of the spores.

Sodium nitrite induced germination of *Clostridium sporogenes* spores [10]. The process was accelerated using increased concentrations of sodium nitrite, a low pH, and a high temperature of incubation. The increase in germination rate with increasing temperature and increasing nitrite concentration may be a reflection in the alteration of the tertiary structure of a spore protein, which in turn may be involved in the calcium–dipicolinic acid complex [10]. The stimulatory effect of nitrite on germination has a dual role in preservation: (i) induction of spores to germinate making them susceptible to a heating process, and (ii) inhibition of the outgrowth of any surviving spores [10]. The lower concentrations inhibited outgrowth of the spore after germination, whereas higher concentrations inhibited germination itself [11]. In the case of *Salmonella*, preservatives (sodium nitrite, pH 6.0; sodium sulfite, pH 6.0; and sodium acetate-citric acid, pH 4.6) in growth media at 22°C can produce biofilm- and virulence-related genes and small RNAs transcription [12]. Nitride exerts a concentration-dependent antimicrobial effect on the outgrowth of spores from *Clostridium botulinum* and other clostridia. The effectiveness of nitride depends on several environmental factors, and the concentration of nitrite required to prevent outgrowth varies with the types of media or foods and environmental conditions.

21.2.2 Factors Affecting the Efficacy of Nitrites

21.2.2.1 Effects of pH

Nitrites have found to be most inhibitory to bacteria at an acidic pH [1]. Tarr [13–15] showed that the preservative action of nitrite in fish was greatly increased by acidification. In a bacteriological medium, the inhibitory action was increased with decreasing pH, particularly at pH 6.0 and below. Grindley [16] suggested that the mode of preservation could be due to the formation of active nitrous acid. Jensen [17] suggested that the increased action of preservation at low pH was due to the undissociated active inhibitor nitrous acid. A tenfold increase in the inhibitory effect of nitride against *Clostridium botulinum* was found when the pH was reduced from 7.0 to 6.0 [18]. A similar tenfold increase for one unit decrease in pH was also observed for *Staphylococcus aureus* [19], *Bacillus* [20], and *Clostridium sporogenes* [21].

The pH dependency of nitrite-induced bacterial inhibition also reflects the conversion of nitrite to nitrous acid [22].

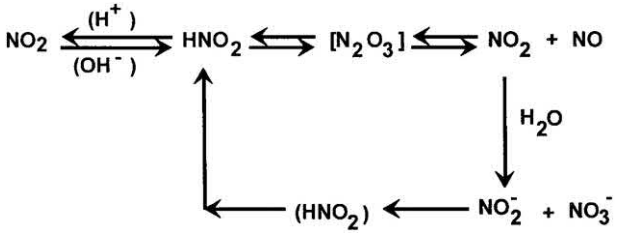

FIGURE 21.1 The dynamics of nitrous acid production in a cyclic reaction. (Adapted from Shank et al. [23].)

Shank et al. [23] mentioned the *nitrite cycle*. The dynamics of nitrous acid production may be visualized in a cyclic reaction where nitrite undergoes a concomitant oxidation–reduction reaction resulting in the formation of nitrate, nitric oxide, and nitrogen dioxide. Nitrogen dioxide reacting with water would generate more nitrate and nitrite with the nitrite reentering the cycle (Figure 21.1). At low pH levels (pH 3 to 4), the cycle rapidly forms NO_3 and NO. At intermediate pH levels (pH 4.5 to 5.5), the cycle rotates more slowly. The presence of HNO_2 is prolonged, thereby increasing its reaction potential. This is the level of maximum bactericidal activity. At higher pH levels (pH 6 to 7), the equilibrium shifts toward $NaNO_2$, the cycle is prevented from functioning, and no bactericidal effects are observed. Nitrous acid and nitric oxide have two fundamental areas of reaction: (i) with the bacterial cell itself, and (ii) with various constituents of the medium making them unavailable for subsequent metabolism. Either or both of these reactions could result in bacteriostasis. Further evidence for bound nitric oxide was presented by Frouin [24]. It was found that all measurable nitrite in various cured products could be volatilized under a high vacuum. In the meat system, nitrite was converted to nitric oxide and may produce a complex equilibrium with other components.

At 20°C, *Clostridium perfringens* growth in laboratory medium was inhibited by 200 mg/L nitrite and 3% salt, or 50 mg/L nitrite and 4% salt at pH 6.2. Fecal *Streptococci* showed growth in the same medium with 400 mg/L and 6% salt [25]. *Salmonella* showed visible growth within 1 week at 20°C in the presence of 400 mg/L nitrite and 4% salt. Significant inhibition by salt and nitrite was achieved only at lower temperatures (10°C or 15°C) and at pH 5.6 or 6.2. *Escherichia coli* was more resistant than *Salmonella*. The inhibition was demonstrated only at the extremes of pH 5.6, salt 6%, nitrite 400 mg/L, and temperature 10°C [26].

Survival of *Listeria monocytogenes* was detected after fermentation and drying, although their number was usually found to be reduced. Surveys of fermented meat products confirmed the presence of *Listeria monocytogenes* in finished products [27]. Junttila et al. [28] concluded that nitrite and nitrate additions to a meat product at officially approved levels did not cause elimination *Listeria monocytogenes*. In broth cultures, acidity and nitrite increased the inactivation rate of *Listeria monocytogenes* [29]. Whiting and Masana [27] studied the effect of nitrite (0–300 mg/L) and pH in uncooked fermented meat products. The time to achieve a 4-log decline as greatly affected by pH, ranged from 21 days at pH 5.0 to less

than 1 day at pH 4.0. Nitrite additions did not affect survival and it was suggested that the effective concentration was the rapidly decreasing residual nitrite level. There is potential for production of bacteriocins by the lactic acid bacteria of the starter cultures in the case of fermented meat [27].

21.2.2.2 Effects of Oxygen

Nitrite provides more inhibitory effects under anaerobic conditions as compared to aerobic [19, 22, 30]. The aerobically cultured *Staphylococcus aureus* were able to grow in the presence of significantly higher concentrations of sodium nitrite than were cultures grown in an aerobic environment [19]. Buchanan and Solberg [22] studied the effect of pH and oxygen pressure on the bacteriostatic accumulation of sodium nitrite toward *Staphylococcus aureus*. They found that the magnitude of inhibition was dependent on the interaction of sodium nitrite concentration, initial pH, and partial pressure of oxygen. Aerobic cultures, after the initial pH decrease, showed a subsequent rise in pH to a level greater than the initial pH, whereas anaerobic cultures remain at the pH level of maximum pH decrease. Injury and cell destruction were most apparent at the lower pH level in the presence of nitrite concentration ≤500 mg/kg. However, 200 mg/kg sodium nitrite in cured meats would offer significant protection against the growth of *Staphylococcus aureus*, particularly if meat product is vacuum packed. Buchanan and Solberg [22] suggested that nitrite may inhibit the growth of *Staphylococcus aureus* by blocking the sulfhydryl sites of either coenzyme A or alpha-lipoic acid, thus blocking the normal metabolism of pyruvate.

21.2.2.3 Effects of Other Food Components

Temperature, salt concentration, and initial inoculum size also significantly influence the antimicrobial role of nitrite [8, 31–34]. It has been reported that sodium chloride alone at concentrations of 9.0% to 10.5% can inhibit growth and toxin production by *Clostridium botulinum*. When nitrite was added in concentrations of 75 and 150 mg/kg, sodium chloride levels of 5.8% and 4.9% were required to inhibit toxin formation. The usual salt levels added to cured meat range from 2% to 3% of the weight of the product. This indicates that salt alone is not always a practical inhibitor of growth and toxin formation by *C. botulinum* [35]. Pierson and Smoot [35] mentioned in his review that the inhibitory effects by the interaction of sodium chloride and nitrite on various bacteria have been widely reported. Riemann [36] found significant inhibition of bacterial spores in a canned meat system by interactions of sodium chloride with sodium nitrite or nitrate as well as pH interactions with sodium chloride. Others also found that inhibition was due to the interaction effects of pH, sodium chloride, and sodium nitrite [37, 38]. Most *C. botulinum* type A and proteolytic type B and F strains would grow in the presence of 150 to 200 mg/kg of sodium nitrite or 6% sodium chloride at pH 6.0, but under the same conditions 200 mg/kg of sodium nitrite plus 3% sodium chloride inhibited almost all of the strains [39]. The combined effects of salt, nitrite, and pH can be synergistic to the inhibition [35].

Roberts et al. [40, 41] studied the combined effect of the following factors on growth of *Clostridium botulinum*: sodium chloride (0–4.5% w/v on water), sodium nitrite (100–300 mg/kg), sodium nitrate (0–500 mg/kg), sodium isoascorbate (0–1000 mg/kg), polyphosphate (0–0.3% w/v), and heat treatment (70–80°C), and storage temperature (15–35°C). Their findings can be summarized as follows:

(i) Increasing nitrite, salt, or heat treatment; adding isoascorbate, polyphosphate, or nitrate; or decreasing storage temperature significantly reduced toxin production.
(ii) The relative effect of increasing nitrite became less in the presence of isoascorbate or high salt levels.
(iii) Increasing salt or heat treatment, adding nitrate, or decreasing storage temperature had less effect if isoascorbate was present.
(iv) The addition of polyphosphate enhanced the effect of adding isoascorbate.

The findings should not be used to assess which combinations give a guaranteed risk of toxin production, since minor changes in product formulation or its production or in experimental conditions might significantly alter the ability to support toxin production and the variability of the system [41].

The combinations of low nitrite (40 mg/kg) plus sorbate/sorbic acid controlled the growth of *Clostridium botulinum* as effective as the level of nitrite (156 mg/kg). The low level of nitrite (40 mg/kg) alone had no significant effect on the growth of *Clostridium botulinum*, but was included to ensure acceptable cured color and flavor [42]. The factors that decreased the toxin production of *Clostridium botulinum* were (i) potassium sorbate, (ii) increasing sodium chloride, (iii) decreasing pH, and (iv) decreasing storage temperature. Heat treatment interacted significantly with some other factors. The effect of sorbate (0.26% w/v) was greater at 3.5% sodium chloride than at 2.5%, at pH values below 6.0, and at low storage temperature [42].

Ethylenediaminetetraacetic (EDTA), isoascorbate, and ascorbate enhance the antibotulinal efficacy of nitrite in canned meat. The degree of inhibition was inversely related to the level of iron and directly related to the level of EDTA. The use of isoascorbate and ascorbate has both positive and negative attributes depending on their level in meat products. At a moderate level, the synergistic effect is due to the sequestering cause of isoascorbate or ascorbate on a cation, iron. On the other hand, excessive levels of ascorbate were shown to decrease the efficacy, because isoascorbate and ascorbate cause more rapid depletion of residual nitrite. EDTA more effectively sequesters iron, thus making iron less available for preventing nitrite inhibition [43]. Tompkin et al. [43] proposed using a minimum of isoascorbate to hasten the curing reaction and stabilize color and flavor, and supplementing with a low level of EDTA for improved botulinal protection. When *Bacillus cereus* was inoculated into uncooked sausage in the presence of 500 mg/kg sodium isoascorbate and 200 mg/kg sodium nitrite and incubated for 48 h at 20°C, no growth was

demonstrated. Sodium isoascorbate alone had no inhibitory effects [44].

21.2.2.4 Effects of Heating

In a bacteriological medium, the inhibitory effect of nitrite is enhanced tenfold after heating due to the formation of an extremely inhibitory substance. This is called the *Perigo effect* [21]. Perigo et al. [21] confirmed that the effect of unheated sodium nitrite was pH dependent and that 200–400 mg/kg sodium nitrite was necessary to inhibit the growth of *Clostridium sporogenes* at pH values around neutrality. They showed that as little as 3–5 ppm sodium nitrite heated in the medium for 20 min at 105–115°C could inhibit growth, and this inhibition was slightly dependent on the pH of the medium. The rate at which this unknown substance is produced was maximal at a temperature of about 110°C. At temperatures exceeding 110°C, the unknown substance appeared to break down or react in such a way that its inhibitory activity declines. Perigo and Roberts [45] confirmed this effect in 30 clostridial strains including *Clostridium botulinum* types A, B, E, and F (14 strains) and *Clostridium welchii* (8 strains). It was reported that a reducing agent such as thioglycollate, ascorbate, or cysteine, and protein hydrolysate were the necessary components of the laboratory medium in order to produce the effect. Roberts and Garcia [46] showed that the inhibition was enhanced due to the Perigo effect in the case of 9 of 14 strains of *Bacillus*. *Streptococcus durans* (*faecium*) was also sensitive to this effect, whereas *Streptococcus faecalis* and *Salmonella* were more resistant.

It has been suggested that one or more new chemical species have been produced [21]. Evidence has been presented that indicates that the media may contain substances such as Roussin's black salt (iron thionitrosyl) [47] and nitrosothiols [48, 49]. Involvement of sulfhydryl groups as well as a nitroso group is probably important [48, 49]. Hansen and Levin [50] mentioned that it may be possible that heat-induced Perigo inhibitors are distinct from these compounds. They suggested that a heat-induced inhibitor presumably of Perigo type was compared with the nitrosothiols of thioglycolate and mercaptoethanol. Phase-contrast microscopy revealed that inhibition of morphological events occurred either before germination or during early outgrowth, depending on inhibitor concentration. The inhibitors derived from nitrite act virtually in every stage of the life cycle of *Bacillus*, suggesting that their mode of action is rather general and that inhibition may be the result of inactivation of several sensitive metabolic systems or steps. A synergistic inhibitory response could help explain the elusive nature of the mode of action of nitrite curing salts as preservatives [50].

An inhibitor of *Clostridium perfringens* formed when low levels of nitrite autoclaved with the defined chemical medium. Only amino acids and mineral salts were involved in the production of this inhibitor. The toxic compound was formed at the sublethal level from cysteine, ferrous sulfate, and sodium nitrite. S-nitrosocysteine, unstable Roussin's red salt, and a complex of cysteine, iron, and nitric oxide were detected. Moran et al. [51] concluded that observed inhibition could be due to the combined effects of sublethal concentrations of each compound. The extended heat treatment of the meat may cause decomposition of the proteins with the liberation of amino acids, peptides, and possibly, amines. Nitrite reacts with amines and amino acids forming N-nitroso compounds, either N-nitosmines or N-nitrosamides, which are toxic and carcinogenic and mutagenic to various species of microorganisms [52].

Johnston et al. [53] found a Perigo-type effect in minced pork. Johnston and Loynes [54] mentioned that the inhibitory effect of nitrite can be increased in the media by the addition of reducing agents, such as cysteine, thioglycolate, and ascorbate. These agents are known to aid in the reduction of nitrite and may affect the formation of nitroso reductants. These intermediate carriers may transfer the nitroso group directly to components of the bacterial cells or release nitric oxide. Johnston and Loynes [54] mentioned that the addition of reducing agents to meat suspensions decreased the redox potential and increased the inhibitory activity by Perigo factor formation. Ashworth and Spencer [55] studied the role of chemical additives in the formation of this inhibition in minced pork and found a similar effect. They added 0.1% of reducing agents in pork slurry containing nitrite and found that its inclusion increased the inhibitory effect of nitrite in the case of sodium ascorbate, cysteine (free base), and thioglycollate, but with sodium formaldehyde sulfoxylate and sodium formaldehyde bisulfite there was a marked decrease in the inhibitory.

Huhtanen and Wasserman [56] suggested that a potent anticlostridial inhibitor can be produced by the addition of iron (ferrous or ferric) without autoclaving nitrite in the medium. They indicated that iron was a limiting factor and sulfhydryl groups were probably necessary for its formation. Similarly, Custer and Hansen [57] found lactoferrin (an iron-binding glycoprotein) and transferrin reacted with nitrite to an inhibitor effective for spore outgrowth of *Bacillus cereus*. The Perigo inhibitor is formed at 105°C or higher, which exceeds the temperatures normally used in the processing of cured meats. Holley [58] mentioned that the Perigo inhibitor is formed in culture medium only when sulfhydryl groups and iron are present. Nitride reacts with various naturally occurring chemical components in the complex system of meat. The heating conditions normally used in the curing process speeds up these reactions, and at the end of the process only about 10–20% of the originally added nitride is analytically detectable. The residual nitride level declines further during storage and distribution [59].

N-nitrosamines, biogenic amines, and residual nitrites are considered as harmful substances, which are often present in cured meats. Initial dry-cured raw sausage contained 5.31 μg/kg of total N-nitrosamines. Cooking by deep-frying or pan-frying resulted in the highest contents (i.e., similar to the raw state) as compared with boiling or microwave treatments. Frying increased N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), and N-nitrosopyrrolidine (NPYR), however it decreased histamine and cadaverine. Cooking (i.e., boiling and microwave treatments) decreased

the total biogenic amines, thus these treatments are suitable methods for cured meat treatments [60].

21.2.2.5 Effect of Irradiation

Pierson and Smoot [35] reviewed the effects of irradiation and mentioned that a limited amount of added nitrite was required to produce acceptable irradiated cured meat products.

21.2.3 Mode of Action to Microflora

A target can be selected from biochemical knowledge in the undesired microorganisms, which is absent from or at least different in the human. This target might be an enzyme or a cellular component involved in a process essential to microbial survival or development [61]. The inhibitory action of sodium nitrite on *Clostridium perfringens* was apparently at the cellular level, since microscopic examination of these organisms indicated no visible difference between inhibited and normal cells. Thus, damage was probably at a submicroscopic level [62]. Yarbrough et al. [63] showed that nitrite has more than one site of attack in the bacterial cell metabolic processes. These are (i) nitrite interferes with energy conservation by inhibiting oxygen uptake, oxidation phosphorylation, and proton dependent active transport; (ii) nitrite acts as an uncoupler, causing a collapse of the proton gradient; and (iii) nitrite inhibits certain metabolic enzymes.

21.2.3.1 Inhibition of the Phosphoroclastic System

In a cell, the oxidation of substrate occurs with concomitant production of adenosine triphosphate (ATP). This can then be used subsequently as an energy source for the synthesis of new cellular material required for growth. In clostridia, an important source of ATP is the oxidation of pyruvate to acetate by the phosphoroclastic system [3]. When nitrite is added to a suspension of cells of *Clostridium sporogenes* incubated in medium containing glucose, there is a large and rapid decrease in the intracellular concentration of ATP and an excretion of pyruvate from the cells [64]. This increase in pyruvate suggested that the phosphoroclastic system is inhibited by nitrite [3]. Iron is required nutrient for clostridial spore germination and outgrowth, and botulinal toxin development. The growth of *Clostridium sporogenes* and *Clostridium botulinum* was inhibited by nitrite through interference with the phosphoroclastic system resulted in an accumulation of pyruvic acid in the medium [64, 65]. The inhibition was due to an interaction occurring between nitrite and intracellular iron-bound protein, i.e., the reaction of nitric oxide with the nonheme iron of pyruvate:ferredoxin oxidoreductase [64]. Nitrite was also shown to inhibit the iron-sulfur enzyme ferredoxin of *Clostridium botulinum* and *Clostridium pasteurianum* [66]. The addition of iron caused a depletion of the residual nitrite levels in cured meats.

The phosphoroclastic system consists of two components: ferredoxin and pyruvate:ferredoxin oxidoreductase. Both of these contain nonheme iron moieties. Pyruvate:ferredoxin oxidoreductase consists of a single protein molecule containing thiamine pyrophosphate and a nonheme iron. Nitric oxide causes inhibition of the phosphoroclastic system by interacting with these components. Nitric oxide is a potent iron ligand that can form coordination complexes with nonheme iron. Pyruvate:ferredoxin oxidoreductase seemed to be more sensitive to nitric oxide [3, 64]. Tompkin et al. [67] also suggested that nitric oxide reacted with iron-containing protein in the cell of *C. botulinum*. Reddy et al. [68] demonstrated the production of iron nitric oxide complexes using electron-spin resonance spectroscopy. The aliphatic and aromatic nitro compounds inhibit the ferredoxin possibly because of formation of S-nitrosothiols by reaction with cysteine residues [69]. Castellani and Niven [19] suggested that the bacteriostatic action of nitrite might be due to interference with the normal metabolism of the hypothetical pyruvate–sulfhydryl complex.

When nitrate and nitrite are added to dry fermented meat sausage, staphylococci experience nitrosative stress [70]. The nitric oxide synthase (NOS) is present in the genome of all staphylococci. NOS produces nitric oxide (NO) and citrulline from arginine. NO is highly reactive with a broad spectrum of activity resulting from targeting metal centers (heme and nonheme) and protein thiols. At low concentration, NO acts as a signaling molecule, while at higher concentration it generates nitrosative stress.

21.2.3.2 Inhibition of Enzyme Systems

At acid pH levels, sodium nitrite exists as nitrous acid, an extremely reactive molecule capable of interaction with a wide variety of substances including myoglobin, ascorbic acid, phenols, secondary amines, amino groups, and thiol groups [71]. Mirna and Hofmann [72] reported that although sodium nitrite reacts with both sulfhydryl (SH) groups and primary amino groups at pH 5.5, the reaction with SH groups is more rapid. Riha and Solberg [62] proposed that nitrite inhibition of *Clostridium perfringens* may be due to a reaction of nitrous acid with SH-containing constituents of the bacterial cell. The nitrite could inhibit enzymes of glucose fermentation such as glyceraldehyde-3-phosphate dehydrogenase and aldolase in *Clostridium perfringens* [71]. Nitrite also inhibited aldolase from *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus faecalis* [63]. Nitrite inhibited the nitrogenase of *Clostridium pasteurianum*, a system comprised of two nonheme-iron-containing proteins. This inhibition was probably due to the reaction of nitric oxide with a component of the nitrogenase system [73].

McMindes and Siedler [74] reported that nitric oxide was the active antimicrobial principle of nitrite and that pyruvate decarboxylase may be an additional target for growth inhibition by nitrite. These observations are substantiated by the fact that the addition of iron to meats containing nitrite reduces the inhibitory effect of the compound [75]. Chelating agents like sodium ascorbate, ethylenediaminetetraacetate, and polyphosphate enhance the antibotulinal action of nitrite. Muscle pigmentation is due to myoglobin and, to a lesser extent, hemoglobin remaining after carcass bleeding. Heart meat showed no inhibition of *Clostridium botulinum* inoculum even with a 156 mg/kg of sodium nitrite added to the product. Adding hemoglobin to the meat formulation reduced

nitrite after processing and decreased botulinal inhibition. The degrees of pigmentation of the meats are roughly grouped in descending order as follows: heart meat, beef round and turkey thigh meat, pork ham, veal, and turkey breast. Tompkin et al. [67] offered the hypothesis that nitric oxide, which was formed from residual nitrite via nitrous acid, reacted with extracellular iron of cells, thereby blocking some metabolic steps essential for outgrowth. The reaction might involve the iron in ferredoxin or an enzyme in which iron played an essential role [67]. The results of Miller and Menichillo [76] demonstrated that use of blood fractions that increased iron levels in beef above 30 mg/kg interfered with the antibotulinal efficacy of sodium nitrite of 156 mg/kg. Lucke [77] observed that blood sausages were associated with foodborne botulism in Germany. It is advisable to include additional microbial growth barriers when iron-containing compounds are added to cured meats [76].

Ingram [78] first postulated that nitrite inactivated enzymes associated with respiration. The active inhibitory agent outside the cell was closely correlated with nitrous acid, while the mechanism of action may vary for different physiological types of microorganism [63]. Nitrite was shown to inhibit active transport, oxygen uptake, and oxidative phosphorylation of *Pseudomonas aeruginosa* by oxidizing ferrous iron of an electron carrier, such as cytochrome oxidase, to the ferric form [79]. Since glucose transport in *Streptococcus faecalis* and *Streptococcus lactis* is not dependent on active transport or cytochromes, nitrite does not inhibit these organisms [79]. Nitrite inhibited the active transport of proline in *Escherichia coli* but not group translocation by the phosphoenolpyruvate:phosphotransferase system [63]. Inhibition of other enzymes, particularly those containing sulfhydryl groups, can occur, but these effects usually occur at higher concentrations of nitrite [3].

There may be damage to the cell wall or membrane indicated by the graying or browning of *C. perfringens* cells incubated with inhibiting concentrations of sodium nitrite [71]. *Staphylococcus faecalis* and *Streptococcus lactis* were highly resistant to nitrite, although aldolase was sensitive to nitrite. This suggested that these streptococci are impermeable to nitrite [63].

21.3 INTERACTION OF NITRITES WITH FOOD COMPONENTS

A water-soluble or low molecular weight compound was responsible for a large part of the nitrite depletion [80, 81]. Sebranek et al. [82] found that nitrite bound to a hot-water-soluble and insoluble meat residue. An amino acid or oligopeptide (probably with an SH group) could be involved in nitrite reduction. Fox and Nicholas [80] examined the effects of various compounds in meat slurries and found that histidine and reductants such as ascorbate and cysteine caused the nitrite depletion. Knowles et al. [83] investigated the interaction of nitrite with bovine serum albumin at gastric pH 2.5, and obtained 3-nitrotyrosine, 3,4-dihydroxyphenylalanine, and 6-hydroxynorleucine. Miwa et al. [84] mentioned that it is well known that primary amino acids react with nitrite to produce alcohol and nitrogen gas (Van Slyke reaction). Among endogenous acidic substances tested, cysteic acid showed the highest ability to decompose nitrite, accompanying the production of unidentified N compounds. Woolford et al. [85] studied the reaction of nitrite with isolated myosin and showed that a part of the lost nitrite was bound to the protein and identified 3-nitrotyrosine as a major product of the reaction. The nitric oxide formed from nitrite may partly bind to protein [72] or ferricytochrome [86]. Frouin [87] concluded that nitrite was rapidly broken down to NO in meat products and reacted with unsaturated carbon–carbon bonds [88]. If whole adipose tissue was treated with nitrite, it was bound to connective tissue, extracted lipid, and unsaturated carbon–carbon bonds. The experiments on various fatty acids and glycerides showed a binding that was apparently related to the degree of unsaturation [89].

Fujimaki et al. [90] studied the fate of nitrite during curing and cooking in the model solutions composed of myoglobin, sodium nitrite, and sodium ascorbate. After curing and cooking, nitrite was recovered as residual nitrite, nitrate, a nitrosyl group of denatured nitrosomyoglobin, and gaseous nitrogen compounds. Almost all of the nitrite was recovered as nitrate whenever greening occurred in the curing period. The gaseous nitrogen compounds were produced under the condition where both sodium nitrite and sodium ascorbate were abundant as compared with myoglobin, and this reaction proceeded not in the curing period but at the cooking stage. The addition of sodium chloride into the model system increased residual nitrite and nitrosomyoglobin [90]. Emi-Miwa et al. [91] also studied the fate of nitrite added to whole meat, meat fractions, and model systems with added sodium ascorbate. They found residue as nitrite, nitrate, nitrosothiol, denatured nitrosomyoglobin and gaseous nitrogen compounds. Twenty percent of the total nitrite lost was changed to nitrosothiol-N in specific interactions between nitrite and sulfhydryl groups of myosin [92]. Olsman [93] also mentioned that more than half of the free nitrite disappeared on the storage of canned cured meat as bound nitrite, probably as nitrosothiols formed with protein-bound thiol groups. The amount of bound nitrite increased with the addition of ferrous ions. This may be due to the formation of ferric coordination complexes between cysteine residues and nitric oxide [94]. Cassens et al. [95] found typical distribution of nitrite in the proportions: myoglobin 5–15%, nitrate 1–10%, nitrite 5–20%, gas 1–5%, sulfhydryl 5–15%, lipid 1–5%, and protein 20–30%.

Namiki and Kada [96] found the formation of ethylnitrolic acid by the reaction of sorbic acid with sodium nitrite by heating at 90°C. The isolated compound ethylnitrolic acid revealed strong activity in comparison with the original materials. The ethylnitrolic acid, sorbic acid, and sodium nitrite were effective at concentrations of 0.025–0.05, 2–4, and 1.5–3 mg/ml, respectively. They also mentioned that ethylnitrolic acid is necessarily formed in foodstuffs containing sorbic acid and sodium nitrite together [96].

Osawa et al. [97] concluded that the main mutagen formed by the nitrite or sorbic acid reaction was 1,4-dinitro-2-methyl pyrole. Piperine was also the mutagen in the nitrite system. The food components, such as ascorbic acid, cysteine, and

some phenolic compounds, were reported to react with nitrite, thus preventing the formation of nitrosamines *in vitro* as well as *in vivo* [98, 99]. Ascorbic acid was reported to inhibit bacterial mutations induced by N-nitroso compounds [100]. The oxidative desmutagenic action of cabbage peroxidase [101] and myeloperoxidase against the mutagenic principles of tryptophan pyrolysate [102] was also reported. The ascorbic acid, cysteine, and other reducing substances were responsible for the desmutagenic action against the mutagens of sorbic acid or a nitrite system.

21.4 FUNCTIONAL AND SENSORY PROPERTIES IMPROVEMENT

Taylor and Sumner [103] mentioned that food additives play an important role in improving health, increasing supply, enhancing appeal, or improving convenience. Among these, health benefits should be given the greatest consideration, while supply benefits are second in importance, and increased convenience and improved appeal are the least important.

Nitrite salts are used for the curing of meat, poultry, and fish products. Curing with nitrite results in the development of a characteristic pink color and distinctive flavor [2]. The sequence of color changes during curing of meat are [3] (i) the initial purple-red color of myoglobin changes to the brown of metmyoglobin; (ii) in reducing conditions nitric oxide derived from nitrite converts this to the dark red nitrosylmyoglobin; and (iii) if the meat is heated, e.g., in cooking, this pigment is converted to the stable nitrosyl hemochrome, which is pink. Ando et al. [104] described that 5′-inosinic acid, adenosine-5′-monophosphoric acid, reduced glutathione, glutamate, and Fe^{2+} influenced the cured color formation.

Nitrite content of 5 mg/kg can produce a satisfactory color for a short time, but it is generally believed that higher concentrations of about 20 mg/kg are necessary for commercial color stability. Nitrite concentrations of at least 50 mg/kg are thought to be necessary for correct flavor development [3, 35]. The addition of nitrite at a minimum level of 50 ppm was necessary to achieve reasonably typical thuringer flavor and appearance characteristics in sliced and baked pizza topping products. At least 100 mg/kg added nitrite was necessary to produce these effects in fried thuringer. The effect of added nitrite above 100 mg/kg was negligible for further color development. The fresh, fried, or baked thuringer containing neither nitrite or nitrate was judged most rancid, and poorest flavor and appearance quality. No nitrosamines were detected in thuringer regardless of initial nitrite (0–150 mg/kg) or nitrate (0–1500 mg/kg) concentration, storage condition, or kitchen preparation method [105]. Pierson and Smoot [35] reviewed the minimum level of nitrite in different food products. These are 20 mg/kg for cured meat and hams, 30 mg/kg for bacon, 25 mg/kg for wieners, 70 mg/kg for pork loins and country style hams, 52 mg/kg for frankfurters, 26 mg/kg for franks, and 40 mg/kg for turkey frankfurters. The modification of the fresh meat flavor is another change produced in meat by the addition of nitrite. A minimum level of 39–50 mg/kg nitrite was required to develop the appropriate flavor [35].

Nitrite upon addition to meat has been associated with the delay of the development of oxidative rancidity [106]. When nitrite reacts with heme compounds to form cured meat pigments, the ferric iron (oxidized state, Fe^{+++}), which is active in lipid oxidation, is reduced to ferrous (Fe^{++}), which is an inactive catalyst [35]. The addition of nitrite to model lipid systems containing Fe^{++} or Fe^{++}-EDTA and aqueous beef extracts substantially reduced oxidation rates [107]. In bacon formulated without or 15 ppm nitrite, off-flavors were found to be high and to increase more rapidly [108]. A significant reduction in the formation of rancid off-flavors in pork during storage was observed when nitrite was added 50 mg/kg or greater [109, 110].

Nitrites and nitrates inhibit dairy cultures by their effect on the activity of a number of oxidoreduction enzymes and consequently the natural ripening of milk is prevented and undesirable microflora is formed [111]. Lactic acid bacteria culture in yogurt has beneficial effects on health. Korenekova et al. [112] studied the effects of nitrites and nitrates on yogurt culture up to a level of 100 mg/kg. They found that nitrites depending on their concentration were able to exert an inhibitory effect on a yogurt culture and nitrates are not marked inhibitors of lactic bacteria. Thus, nitrates can be used in yogurt to preserve its quality without inhibiting lactic acid bacteria.

21.5 MEDICAL OR HEALTH ASPECTS

Two types of health benefits may be provided by food additives and food components: (i) those that prevent or reduce the incidence of specific diseases, and (ii) those that provide enhanced nutrition [103]. It is a common attempt to develop an agent that inhibits or inactivates the undesired organisms but displays little toxicity toward humans when ingested [61].

The National Academy of Sciences [113] concluded that 39% of dietary nitrite intake was from cured meat, 34% from baked goods and cereals, and 16% from vegetables. Cassens [114] mentioned that nitrate is important in the total picture because it is found in substantial quantities in other foods such as green leafy vegetables and root vegetables and sometimes in drinking water. They observed no detectable nitrate in cured meats in their studies.

Prolonged ingestion of sodium nitrite or sodium nitrate has shown to cause methemoglobinemia, especially in infants. Methemoglobinemia causes production of abnormal hemoglobin [113]. The major adverse effect of nitrites is the possible induction of cancer. In rats it increases the incidence of lymphoma when fed 250–2000 mg/kg nitrite in their food or water [115]. Nitrite results in the formation of carcinogenic N-nitrosamines with secondary amines or with substitutes amides to form nitrosamides. Over 65 different nitrosamines detected in a variety of foods, including cheese, meats, mushrooms, and alcoholic beverages, have been found to be carcinogenic [116]. Epidemiological studies have indicated a possible link between exposure to high levels of nitrites and a high incidence of stomach and esophageal cancer [113, 117]. Another well-known effect of nitrite is the lowering of oxygen

transport by the bloodstream through the mechanism of oxidizing hemoglobin to methemoglobin [35]. Thus, the nitrite level should be reduced in cured products. Ascorbates or erythorbates are added to reduce nitrosamine formation [2].

An oral challenge test with 30 mg of sodium nitrite may cause urticaria, intestinal disorders, or headache [118, 119]. It may cause cellular anoxia and inhibit the protective enzymatic activities of the intestinal mucosa. This may lead to increased permeability of the mucosa to other antigens. In addition, sodium nitrite some way or another enhances the effect of histamine present in many foods [120].

The lethal dose of nitrites in humans is 32 mg/kg body weight or 2 g [121] and 4–6 g [122]. In 1973, the U.S. Department of Agriculture (USDA) established an expert panel on nitrates, nitrites, and nitrosamine. It concluded that (i) the use of sodium nitrate should be discontinued in all meat and poultry products; (ii) the nitrite level permitted for curing of meat should be limited to 156 mg/kg in canned, cured sterile products; (iii) the permitted residual nitrite level should be reduced from 200 to 100 mg/kg in cooked sausage products, 125 mg/kg in canned and pickle cured products, and 50 mg/kg in canned cured sterile products [113]; (iv) sodium nitrite (120 mg/kg) and potassium nitrite (140 mg/kg) be added to bacon along with sodium ascorbate or erythorbate (550 mg/kg) to assist in the prevention of nitrosamine formation [117]. The regulations in 1986 for nitrite in bacon allow one of the following: (i) 120 mg/kg sodium nitrite or 148 mg/kg potassium nitrite plus 550 mg/kg sodium erythorbate or isoascorbate, (ii) 100 mg/kg sodium nitrite or 123 mg/kg potassium nitrite plus 550 mg/kg sodium erythorbate or isoascorbate if a demonstration of adequate process control is met, or (iii) 40–80 mg/kg sodium nitrite or 49–99 mg/kg potassium nitrite plus 550 mg/kg sodium erythorbate or isoascorbate plus 0.7% sucrose and a lactic acid bacterial culture (*Pediococcus*). The level of nitrites allowed is a maximum of 10 ppm in smoked cured tuna fish and 200 mg/kg (input not to exceed 500 mg/kg) in smoked cured stable fish, salmon, shad, cod roe, and in-home curing mixtures. The level in smoked chub is fixed at 100–200 mg/kg. The use of nitrite in other products is limited to a maximum residual level of 200 mg/kg [1].

The product development efforts have resulted in an entire new generation of cured meat products, which are low in fat and formulated with ingredients not previously used [123]. White [124] reported an average residual nitrite in cured meats of 52.5 ppm and a range of 0–195 ppm residual nitride in wieners. Recently, Cassens [114] found 5, 10, and 15 mg/kg residual nitrite on various cured meats in three trials of 164 samples. It is a reasonable conclusion that the current residual nitrite content of cured meats sold at retail in the United States is approximately 10 mg/kg. Cassens [114] mentioned that this change has undoubtedly resulted from (i) lowered ingoing nitrite, (ii) increased use of ascorbates, (iii) improved process control, and (iv) altered formulation. The mean value for the residual ascorbates was 209 mg/kg, which was nearly 40% of the maximum allowable addition of 550 mg/kg. The ascorbates routinely used are ascorbic acid, sodium ascorbate, erythorbic acid, and sodium erythorbate. Mirvish et al. [125] showed intragastric formation of N-nitrosamines in humans with higher doses of nitrate, but ascorbic acid inhibited their formation.

Nitric oxide is synthesized in the human body and is important to several physiological functions [114]. Cassens [114] mentioned that nitrite and its reaction products are important in human physiology. It is known that nitric oxide is formed in human body from nitrite. He reviewed the benefits of nitric oxide, which are (i) it is a biological messenger important to the physiological functions of neurotransmission, blood clotting, blood pressure control, and immune system function; and (ii) generation of salivary nitrite from dietary nitrate may also provide significant protection against gut pathogens in humans.

In some cases, the comparative risks are obvious. The risk of using nitrites and acquiring cancer from exposure to nitrosamines must be balanced against the risk of not using nitrites and acquiring botulism from cured meat. The comparative risks are more obscure or difficult to quantify [103].

21.6 POSSIBLE ALTERNATIVE TO NITRITE

Consumers are now demanding more organic and natural meat products due to the health risk of nitrite and nitrate [126]. It is difficult to find any single compound that performs all functions of nitrite, although focus was given to the alternatives to nitrite in meat products [127]. Therefore, nitrate and nitrite are still common additives in the meat industry.

21.6.1 Effects of Nitrite Reduction

The reduction of nitrite and nitrate affects the types of bacterial growth, and volatile formation in dry fermented sausages (i.e., maximum amount allowed by the European Union: combination of 150 mg/kg KNO_3 and 150 mg/kg $NaNO_2$, 50% and 25% reduction and control). There was a relation between ingoing and residual nitrite, which was 3.5-fold higher when the maximum amount was used in comparison to the 50% reduction [127]. The concentration affected gram-positive catalase-positive cocci (which numbers were 1 and 2 log cfu/g higher in the 50% reduction and control batches, respectively) and Enterobacteriaceae. A higher amount of volatiles derived from amino acid degradation, and carbohydrate fermentation was related to the microbiological changes [127].

Volatiles and aroma development in meat products is a complex process of many factors and their interactions. These include raw material and ingredients by contributing to major precursors (proteins and lipids), presence of additives (salt and curing agents), and methods of processing (i.e., wet, dry cured, and fermented) [128]. The cured odor is a balance between aldehyde compounds (green odor notes) formed during oxidation reactions and sulfur, key odor compounds producing meaty odors [129, 130]. In wet meat products, the main reactions for aroma development are lipid degradation (oxidative reactions), Maillard reactions, Strecker degradation, and thiamine degradation, while in dry meat products the reactions are lipid degradation (oxidative reactions), thiamine

degradation, microbial carbohydrate fermentation, and microbial metabolism including complex interactions (such as the amino acid degradation produced by lipid oxidation products) [131]. Nitrite sausages showed high aroma values for ethanol, 1-hexanol, propanoic acid, (E)-2-heptenal, and nonanal, while nitrate sausages showed high aroma values for phenylacetaldehyde and 3-methylbutanal [132]. In addition, fermentation conditions of fast or slow also affect the aroma profile [133].

In the case of slow fermented sausage, Perea-Sanz et al. [134] studied the effects of ongoing nitrite (i.e., control, 250 mg/kg; 15% and 25% reduction) on microbiology and chemical parameters, volatile compounds, and aroma production. The pH, water activity, and color decreased during ripening, without being affected by nitrate reduction. Lipid oxidation increased during ripening and it was higher in control sausages; residual nitrate decreased during ripening, with higher reduction in 25% decreased sausages. The fermentation time mainly influenced microbial counts and 15% reduction did not affect the microbial counts, while 25% reduction caused slightly different yields in yeasts and molds. Nitrate reduction resulted in an increase of volatile compounds derived mainly from amino acid degradation, and lesser extent esterase activity; key aroma compounds altered the sausage aroma profile. The affected microbial metabolism can cause nitrate reduction into nitrite and hence its effect on lipid oxidation. In the case of dry fermented sausages (i.e., different levels of reduction of nitrite and nitrate), Hospital et al. [135] identified that environmental factors such as pH, a_w, and the competitive microbiota could exert a more relevant role than nitrite in the inhibition of the growth and toxin production by *Clostridium botulinum*, therefore all technological factors need to be carefully considered.

The growth of *Listeria* and *Salmonella* in French dry fermented sausage was affected by the nitrate/nitrite formulation [136]. In the presence of nitrate alone, despite the hurdles (low water activity, pH. and salt level), both pathogens could not be inhibited due to the slow conversion of nitrate to nitrite, thus nitrite was a relevant hurdle. A reduction of 47% nitrite provided the same effect as the regulatory dose (i.e., 250 mg/kg sodium nitrate or 150 and 150 mg/kg of sodium nitrate and sodium nitrate). This is similar to the guidelines of the Food Chain Evaluation Consortium, which concluded that the range of 80 to 100 mg/kg sodium nitrite added in sausage formulation would be reasonably safe for a majority of products when used in combination with other hurdles [137].

Majou and Christieans [138] reviewed the mechanisms of the bactericidal effects of nitrate and nitrite in cured meats. In this system, anaerobic and acidic conditions are the most effective parameters at different stages. The bacterial stress is highly pH-dependent and non-oxygen-dependent, and enhanced by the nitrate–nitrite–peroxynitrite (ONOO–) system (strong oxidant). When homeostasis is unbalanced by acidity and anaerobic condition, nitrate and nitrite have a synergistic and aggravating effect depending on their concentration. Other hurdles, such as sodium chloride, ascorbate, and water activity enhance or reduce stress (i.e., effectiveness) on the bacterial system. The most resistant are gram-negative aerobic/facultative anaerobic bacteria (*Escherichia coli*, *Salmonella*), and the most fragile are gram-positive anaerobic bacteria (*Clostridium botulinum*).

21.6.2 Alternatives to Nitrite

Alahakoon et al. [126] reviewed the potential alternatives to replace (i.e., partial and complete) nitrite salts in meat products. They grouped the alternatives into use of plant materials, organic acids, microbial source compounds (such as bacteriocin and nisin), and additional hurdles, such as high pressure.

21.6.2.1 Natural Extracts or Materials

Carotenoprotein from the shells of blue crabs was incorporated to improve the quality and the shelf life of reduced-nitrites turkey meat sausages [139]. It exhibited antioxidant activity (i.e., degree of lipid oxidation) in a dose-dependent manner (i.e., inhibited thiobarbituric acid substances, TBARS, and conjugated dienes formation) and antimicrobial potential (i.e., shelf life of 10 days based on total mesophilic flora, at 4°C). It also showed scavenging effects of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, inhibited myoglobin oxidation inhibition, improved the transformation of the heme iron and lower free fatty acids (FFAs) contents, and improved stability in the color parameters.

Vegetables such as celery, spinach, radish, and lettuce have been reported to contain more than 2500 mg nitrate/kg [140]. Plant-based curing ingredients could also meet the demand for natural products. The effects of celery juice powder as a natural source of nitrite and reducing agents (i.e., no reducing compound or sodium erythorbate/ascorbic acid from cherry powder) were compared with the sodium nitrite considering color development and residual nitrite in a cured meat model system [141]. Only sodium nitrite (i.e., 156 mg/kg) showed 74.7 mg/kg pigments and 79.7 mg/kg residual nitrite, while addition of the reducing agent sodium erythorbate (495 mg/kg) showed 130.9 mg/kg pigments and 62.6 mg/kg residual nitrite. Celery juice powder (0.44% addition provides 100 mg/kg sodium nitrite) alone contained 51.9 mg/kg pigments and 59.3 mg/kg residual nitrite, while addition of 440 ppm of ascorbic acid from 0.4% cherry powder showed 124.8 and 31.6 mg/kg, respectively. The treatments with reducing compounds showed greater cured pigment, displayed increased redness and decreased yellowness, and reduced residual nitrite. However, chemical sodium nitrite treatments showed greater cured meat pigment, increased redness, and decreased yellowness. Therefore, it is important to include a reducing agent when using plant-based curing ingredients that contain lower amounts of ingoing nitrite to produce a product similar those cured with sodium nitrite and sodium erythorbate.

The beetroot powder (0.12% beetroot powder and 100 mg/kg sodium nitrite, 0.24% beetroot powder and 50 mg/kg sodium nitrite; and 0.35% beetroot powder) in Turkish fermented beef sausage (sujuk) was used as a nitrite (150 mg/kg) alternative [142]. The lactic acid bacteria count was highest in the case of 0.35% beetroot powder, and sensory evaluation scores were comparable with the control. Considering

oxidation (i.e., thiobarbituric acid reactive substance [TBARS] value), a 56-day storage period was suggested for the with no or low sodium nitrite, while 84 days at 4°C for others. Two mixtures of natural antioxidants—(i) grape seed extract and olive pomace hydroxytyrosol (GSE) and (ii) chestnut extract and olive pomace hydroxytyrosol (CHE)—proved effective in nitrite-replaced dry fermented pork sausages [143]. *Listeria monocytogenes*, *Salmonella*, and *Clostridium botulinum* were not found, whereas replaced additives showed a slightly lower antioxidant activity. The volatile profile showed a similar aromatic profile among the three treatments for overall acceptability, except that color-related traits were underscored in the sausage with replaced ingredients. Lactic acid is currently permitted as an additive in the production of organic foodstuffs of either plant or animal origin, with no specific upper limit of usage [126]. Bacteriocins and nisin are also commonly used as alternative or additional hurdles.

21.6.2.2 Organic Acids

Lactate, sorbate, citrate, and benzoate are used to reduce the nitrite level in meat products [126]. Lactate (organic acid) is used as an antimicrobial agent in meat systems. It also enhances meat flavor owing to its salty taste [144], improves color stability [145, 146], and imparts its taste buffering ability and humectant properties [147]. The improved color stability may be due to replenishing of the reduced form of nicotinamide adenine dinucleotide (NADH) when lactate is converted to pyruvate by lactate dehydrogenase. This increased the metmyoglobin-reducing activity. In addition, deoxymyoglobin can convert nitrite to nitric oxide, thus the production of more nitric oxide caused reduced residual nitrite levels [148]. Lactate shows the effectiveness in inhibitory activity against aerobic and anaerobic microorganisms in meat [146]. It showed inhibitory effects on different strains of *Clostridium Botulinum* [149, 150] and *Listeria* [151, 152].

21.6.2.3 Other Hurdles

The combination of high pressure with another hurdle technique can result in a synergy between the different hurdles and thereby possibly be used with nitrite. The combined effect of high pressure (up to 500 MPa at 20°C for 6 min) with sodium chloride (0–3 mg/kg) or sodium nitrite (0–100 mg/kg) on the outgrowth of endogenous flora of pork meat, including aerobic mesophiles, lactic acid bacteria, and Enterobacteriaceae members showed that 350 MPa or salt alone was not sufficient to delay the growth [153]. However, a combination of high pressure with 1.5 and 3 mg/kg of salt was found to reduce the microbial counts of less than 2 log cfu/g. High pressure 600 MPa for 3 min in combination with nitrite resulted in an immediate reduction of 3.9–4.3 log cfu/g in the *Listeria monocytogenes* populations in ready-to-eat sliced ham [154]. More examples of the combined effects of high pressure and nitrite are given by Alahakoon et al. [126].

21.7 CONCLUSION

Nitrite and nitrate are mainly used to maintain microbial quality, flavor, and color, and to prevent lipid oxidation. The addition of 200–400 mg/kg is commonly effective for microbial growth and toxin production. The factors affecting their efficacy are pH, aerobic or anaerobic conditions, salts, acids, and compositions of the products. Because consumers now demand organic or natural meat products due to the health risk of synthetic additives, the meat industry is currently focusing on the development of nitrite alternatives. The alternatives, such as natural extracts and biomaterials, acids, and other hurdles (such as high pressure) are being attempted. However, replacing the nitrite should be carefully applied considering the risk–benefit balance between chemical and microbiological hazards.

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Part IV

Preservation by Controlling Water, Structure, and Atmosphere

22 Modified-Atmosphere Packaging of Produce*

Leon G. M. Gorris and Herman W. Peppelenbos

CONTENTS

22.1 MODIFIED-ATMOSPHERE PACKAGING: RATIONALE*

Immediately after harvest, the sensorial, nutritional, and organoleptic quality of fresh produce will start to decline as a result of altered plant metabolism and microbial growth. This quality deterioration is the result of produce transpiration, senescence, ripening-associated processes, wound-initiated reactions, and the development of postharvest disorders. In addition, microbial proliferation contributes markedly to postharvest quality loss. The relative importance of individual deterioration processes in determining the end of the shelf life will depend upon specific product characteristics as well as upon external factors. Low temperature and proper hygienic handling of the material are the prime factors that control these processes. In addition, modified-atmosphere packaging (MAP) is a preservation technique that may further minimize the physiological and microbial decay of perishable produce by keeping them in an atmosphere that is different from the normal composition of air [1–6].

* This chapter has not been updated from the first edition.

MAP of respiring food products such as fresh and minimally processed produce requires a different approach than MAP of nonrespiring foods. With nonrespiring foods, modified atmospheres without oxygen are used to minimize oxidative deterioration reactions, such as brown discoloration of meat or rancidity of peanuts, or to reduce microbial proliferation, e.g., the growth of molds in cheese and bakery products. High gas barrier films or laminates are used to exclude the exchange of gases (especially O_2) through the package, which would result in a less beneficial in-package gas atmosphere. In contrast, respiring products stay metabolically active after harvest, and this activity is essential for keeping their quality.

Aiming at the extension of the shelf life of respiring products through MAP, a prerequisite for a suitable packaging system will be that the composition of the gas atmosphere allows for a basic level of metabolism, which means that a certain amount of O_2 should be available. The required basic level of metabolism is highly at variance with different commodities (type, maturity) and heavily depends on the storage temperature and the degree of processing (trimming, cutting, slicing, etc.) applied. Due to the significant respiratory activity of the product, the gas atmosphere inside the package changes during the course of the storage period, and expert knowledge about these changes is necessary to tailor the package design of an individual product to optimize quality shelf life.

MAP of fresh and minimally processed fruits and vegetables is a preservation system that is nonsterile by design. Fruits and vegetables are characterized by an elaborate microflora, consisting of many different types of bacteria, molds, and yeasts, most of which are involved in the spoilage of produce but are harmless to the human consumer. Microorganisms that are dangerous to humans (pathogens that are toxic or cause infectious diseases) normally cannot establish a dangerous population density because they have to compete with the spoilage microflora. However, packaging the produce will change the microenvironment perceived by the microorganisms and may well impair this safe balance. Consequently, evaluation of the impact of package design and use in the logistic chain is a mandatory exercise to ensure consumer safety.

22.2 EARLY RESEARCH ON MODIFIED-ATMOSPHERE PACKAGING

Packaging techniques based on altered gas conditions have a long history. Ancient Chinese writings report the transport of fruits in sealed clay pots with fresh leaves and grass added. The respiratory activity of the various plant products generated a low oxygen and high carbon dioxide atmosphere, which retarded the ripening of the fruit [7, 8]. At the beginning of the 19th century Berard demonstrated that fruit placed in closed containers did not ripen [9]. By the end of the 19th century, the first patent was granted covering the use of a CO_2/CO mixture to extend the shelf life of meat [3]. Extensive research on the use of altered gas conditions for fruits tailed early in the 20th century, with the work of Kidd and West [10]. Commercial storage under altered gas conditions was undertaken in England in 1929 when apples were stored in 10% CO_2 and ambient O_2 [11]. Reduced O_2 concentrations and increased CO_2 concentrations also proved to be beneficial for harvested products other than apples. Products with a high potential for a successful commercial application in MAP include apple, banana, broccoli, cabbage, cherry, chicory, and brussels sprouts. The first commercial application of MAP did not take place until 1974 when the technique was used for meat [3]. The use of modified atmospheres (MA) for storage and packaging has increased steadily over the years and contributed strongly to extending the postharvest life and maintaining the quality of fruits and vegetables [12]. In fact, the biggest growth in the use of MA has been for fresh fruits and vegetables, especially for minimally processed salads [3]. The technique of MA is now applied at a range of different sizes, i.e., for bulk storage packages (e.g., red currants), transport packages (e.g., bananas, strawberries), and consumer packages (e.g., apples, broccoli).

22.3 EFFECTS OF MODIFIED GAS ATMOSPHERES

The strategy of packaging produce under modified atmospheres is to slow down the metabolic activity of the product as well as of the growth of microorganisms (both spoilage and pathogenic) present by limiting O_2 supply and by application of an elevated level of CO_2. Because the same strategy underlies refrigerated storage, MAP of respiring produce is usually combined with this technique. Many commodities, for instance, avocados, mangoes, papayas, and cucumbers, are very sensitive to low-temperature injury and should not be stored below about 13°C. Commodities like apples, broccoli, and pears are not sensitive to chilling and can be stored near 0°C without ill effect [13].

22.3.1 Reduction of Oxidative Reactions

Plant parts such as seeds, fruits, leaves, or roots continue to live after harvest. The energy plant cells need to stay alive and/or to proceed with ripening is generated by aerobic respiratory processes. Respiration involves the consumption of O_2 and the production of CO_2. A reduction of respiration results in a lower energy supply and a reduced rate of changes within the product, like ripening [5]. To extend storage periods, conditions should be created that reduce respiration, for instance, by using low-temperature and low-O_2 concentrations. In general, the reduction of the respiration rate is regarded to be the process that is most strongly affected by altered gas conditions [14, 15]. For certain fruits, low O_2 levels inhibit the production and action of the plant hormone ethylene, which results in reduced ripening as well [2]. Because respiration has such an important central position in the overall metabolism of a plant (part), its measurement is often used as a general measure of metabolic rate. Specific metabolic changes, however, may occur without measurable changes in net respiration [11].

Nevertheless, good quantification of the effect of reduced O_2 on respiration rates is essential for MA, as this process helps to generate the modified atmosphere inside MA packages.

With both whole, fresh produce and minimally processed produce, oxidative reactions do not only relate to respiratory activity. In addition, oxygen also has an effect on the activity of certain enzymes present in bruised or wounded tissue. Such enzymes are involved in wound repair reactions and in the defense against intruding microorganisms. Their activity depends on the presence of oxygen and is driven by the metabolic activity of the produce. Most studied is polyphenol oxidase (PPO), an enzyme that causes browning of plant tissues. In the case of minimally processed products (i.e., chopped, cut, sliced, and peeled), the level of tissue injury is much higher than with whole produce. Consequently, the level of metabolic activity and thus the respiration rate of minimally processed produce is often orders of magnitude higher than that of the raw material. Also, enzymes such as PPO will be more active and may cause visible browning of cut surfaces. Such responses should be considered and overcome by choosing the correct MAP design. In the case that different types of minimally processed products are included in a MAP, which is often the case in mixed vegetable salads, conflicting levels of O_2 and CO_2 may be optimal for the individual components. A designer's solution for this problem needs to integrate all the different aspects that are important with regard to the quality features of the end product.

22.3.2 Fermentation Reactions

The most optimal MA condition for a product is often considered to be the O_2 concentration, which is as low as possible with regard to product respiration without initiating fermentative reactions [2, 14]. Fermentative reactions lead to the production of compounds such as acetaldehyde, ethanol, lactic acid, and ethyl acetate. Alcoholic fermentation is always found in plant tissues exposed to an environment without O_2 [16]. An increased concentration of ethanol and/or ethyl acetate is often related to quality problems such as off-taste and off-odor [17, 18]. A strong correlation was found between ethanol and ethyl acetate concentrations [17]. A relationship between other fermentative metabolites and off-flavors is less clear. With improved detection techniques, compounds like ethanol and acetaldehyde can even be detected at O_2 concentrations higher than those considered to be optimal for the packaging of certain produce [19]. It seems that fermentation cannot be completely avoided and that it is not absolutely necessary to be avoided from the point of view of package design. Rather, it is important at what concentration of ethanol (or ethyl acetate) the consumer experiences off-odors or off-flavors. The package design should allow O_2 concentrations to be high enough to avoid an accumulation to that concentration. A complicating factor is that a relatively short period of too low O_2 concentrations can cause irreversible quality damage, because it has been found that strong off-flavors do not disappear once favorable O_2 levels have been reestablished.

22.3.3 Selective Impact on Microbial Growth

For many minimally processed products, the main factor causing quality loss is not ripening or senescence, but microbial growth. The modified-atmosphere composition has a marked impact on the growth of spoilage microorganisms as well as on pathogens that occasionally occur in minimally processed produce [20]. The very low O_2 (typically 2–3%) and moderately high CO_2 (5–20%) levels prevailing inside a package slow the proliferation of aerobic spoilage microorganisms [4, 6, 8, 21–25]. The antimicrobial effect of CO_2 on microorganisms has been intensively documented [26–35]. However, it has been shown recently that only CO_2 levels well above 20–50% significantly affect the growth of psychrotrophic pathogens that are relevant to MA-packaged produce [36]. This contradicts the general belief that CO_2 has very pronounced antimicrobial properties. At levels of O_2 and CO_2 that are generally favorable for storage of produce, there is certainly no beneficial effect of CO_2 [36, 37].

In *in situ* studies, it was established that the specific conditions of MAP (reduced oxygen, increased carbon dioxide) can lead to marked changes in the epiphytic microflora, especially in chicory endive [38]. Thus, whereas there may be no direct antimicrobial effect of CO_2, there is an influence on the composition of the microflora and on the competition that pathogens may experience in this ecosystem. A specific safety hazard is that psychrotrophic, facultative aerobic pathogens such as *Listeria monocytogenes* are not suppressed under MA conditions that are optimal for respiring produce [36, 37, 39, 40]. On the contrary, growth may be enhanced in certain cases [38, 41], especially because the MA conditions diminish the growth of spoilage microorganisms that would be competitors of the pathogens.

22.4 TYPES OF PACKAGES

With MAP, the gas composition surrounding the produce inside the package is different from the gas composition outside the package. Outside, the gas composition is always close to 78.1 kPa nitrogen, 20.95 kPa oxygen, 0.93 kPa argon, and 0.036 kPa carbon dioxide. Several different types of packages and packaging techniques have been developed to accommodate modified atmospheres around the produce, and these will be explained in detail next. The modification of the atmosphere generally implies a reduction of O_2 content and/or an increase of the CO_2 concentration, but in some cases changing the level of carbon monoxide (CO), ethylene, ethanol, or other compounds in the atmosphere can also contribute to shelf-life extension. Modified atmospheres can be created passively by the respiration activity of the product inside the package (product-modified MAP) or actively by introducing the desired gas mixture (gas packing). Other active ways of obtaining modified atmospheres are the use of gas generators and scrubbers (controlled-atmosphere packaging), evacuation of air (hypobaric storage, vacuum packaging), or addition of chemical systems that absorb or generate gases or volatile compounds (active packaging) in packages.

22.4.1 Modified-Atmosphere Packaging

In modified-atmosphere packaging the gas composition within the package is not monitored or adjusted. Therefore, the term passive atmosphere packaging (PAP) is sometimes used in this respect. Depending on the oxygen sensitivity and metabolic activity of the product to be packaged, air or a predetermined gas mixture is used to flush packages before closing. The use of ambient air as the packaging gas obviously is most economical, but is an option mainly when the respiration activity under the prevailing storage conditions is high enough to reduce the in-pack O_2 level fast enough to lower levels that do not cause physiological or microbial deterioration. With produce highly sensitive to O_2 (e.g., many minimally processed fruits) or that have a low level of respiratory activity, flushing with a gas mixture composed of low oxygen and moderately high CO_2 is often used to shorten the time needed to reach the desired in-pack gas composition. After closing the package, the respiration of the product will cause a decrease in the oxygen content and an increase in the carbon dioxide content. These altered gas concentrations, however, cause a decrease in the respiration rate. Finally, an equilibrium concentration inside the package is reached, which is the result of a balance between metabolic rates of the packed product and diffusion characteristics of the package materials. This explains the use of another term, equilibrium-modified atmosphere (EMA) packaging. The package is often designed in such a way that the equilibrium concentrations resemble the optimal gas concentrations found in experiments where products are stored under a range of stable gas conditions.

The course of the atmosphere modification is determined by three interacting processes: respiration of the commodity, gas diffusion through the commodity, and gas permeation through the film. Each of these processes is in turn strongly influenced by several commodity- and environment-generated factors. Respiration of a certain commodity depends, among others, on its physiological stage and temperature, O_2 and CO_2 partial pressures, relative humidity, and ethylene concentration. Gas diffusion is affected by temperature; the gas gradient across the limiting barrier; and the commodity's mass, volume, respiration rate, membrane permeability, and gas diffusion path. Some of these variables may vary with the maturity stage of the product or even the degree of illumination. Some variables affecting gas permeation through the film are temperature, gas gradient across the film and film structure, water vapor gradient, thickness, and surface area. A change in product amount, free volume, or any of the aforementioned variables will affect the EMA and/or the time in which the steady-state conditions are established. Flushing a package with a premixed gas will influence the time needed to attain the EMA.

Strict temperature control in the distribution chain would be a prerequisite for optimal use of MAP in practice, but in most countries, the cooling chain between production, distribution, retail, and the consumer has many uncontrolled links. The changes in the permeabilities of most packaging films to gases in response to changes in temperature are generally lower than changes in product respiration. Most of today's existing plastic films do not have the proper O_2:CO_2 permeability ratio to provide the ideal MA for many commodities at a given temperature. In view of all these variables and knowing that any change within or around the package will alter the dynamic equilibrium between the product and its environment, it is clear that knowledge about the limits of tolerance of a certain commodity is even more important for MAP than it is for controlled-atmosphere packaging.

22.4.2 Controlled-Atmosphere Packaging

In controlled-atmosphere packaging (CAP) the altered gas composition inside the package is monitored and maintained at a preset level by means of scrubbers and the inlet of gases. This method closely resembles the practices used in large controlled-atmosphere (CA) storage facilities where produce is stored essentially unpacked in bulk, except that CAP is used for storage or transport of smaller quantities of produce.

Additionally, new areas of attention in CA storage today are ultra-low oxygen (ULO) storage and dynamic CA storage. Obviously, these techniques can be used in CAP as well. ULO storage uses O_2 levels close to the minimum level required for maintenance of plant tissues; lower levels will induce disorders such as browning and tissue necrosis. Using ULO storage at 1–2°C with preset levels of 0.5–1% O_2 and 2–3% CO_2, for instance, Elstar apples can be stored for almost a whole year without unacceptable quality loss. In the case of dynamic CA storage, sometimes referred to as interactive CA storage, gas levels are not controlled at preset levels but are continuously adapted to the physiological response of the produce [42], for instance by monitoring fermentation products or cell degradation products. In this way, an optimal match is made between the physiological demand and tolerance of a product and the storage conditions. Although this concept is still in development for CA packages [43], comparable ideas have been described (see later).

22.4.3 Active Packaging

In some cases, a package cannot be designed in such a way that optimal conditions will be reached passively. "Active packaging" can then provide a solution, by adding materials that absorb or release a specific compound in the gas phase. Compounds that can be absorbed are carbon dioxide, oxygen, water vapor, ethylene, or volatiles that influence taste and aroma. For some leafy vegetables, carbon dioxide levels can induce browning of tissues, while for most fruits increased ethylene levels cause an acceleration of ripening. Even at rather low levels, depending on the type of produce, ethylene can induce senescence and maturation processes that reduce the fresh product quality. The inclusion of ethylene scrubbers like potassium permanganate counteracts the effect of ethylene, although the capacity of such scavengers is finite. In transport packages for grapes pouches are often added that slowly release sulfur-containing chemicals to reduce fungal growth. Recently research has been directed to replacing chemicals

with compounds retrieved from plant tissues ("green chemicals"). How much of the active compounds needs to be added will depend on a range of interacting factors, such as production rates (carbon dioxide, ethylene), concentrations to be reached, and how long the package should be functional. Various possibilities exist, although precise control of O_2 in such packages is not possible [2].

Recently a number of new "intelligent" concepts have been introduced that involve more than only scrubbing or emitting compounds. These types of packages will only become "active" when a specific prerequisite has been met. Most of these packages focus on the prevention of problems associated with anaerobic conditions. In one such system, holes are introduced in the package upon exposure to high temperatures for a certain time; originally, the holes are closed by solid hydrocarbons that have melting points between 10°C and 30°C [2]. Because respiration of a product often increases at a faster rate than the diffusion of gases with a rise in temperatures, the holes in the package will prevent the depletion of O_2. Another idea is a sensor for ethanol mounted on a package that informs possible buyers of the history of the package in terms of possible mechanical damage or temperature abuse [2]. Yet another concept that has seen some use in, for instance, France and the United States, is the "time–temperature indicator" or "time–temperature integrator" (TTI). TTIs used now are in most cases small devices that, attached to the package, will indicate the combined time and temperature history of that product by a gradual change in color [44, 45]. TTIs integrate the time and temperature by specific enzymatic or chemical reactions that, ideally, have an identical rate constant to the quality or safety feature of the packed product. The consumer can compare the actual color at the time of intended purchase with the indicated sell-by limit color. A TTI is an elegant and user-friendly improvement that informs consumers of the expected shelf life at the point of sale. The concept could well be extended to the home situation.

22.4.4 Vacuum Packaging

Whereas MAP and CAP mostly operate at ambient pressure (101 kPa), storage at reduced atmospheric pressures has been experimented with and, in some cases, has been used for bulk storage (e.g., in the so-called hypobaric storage systems designed by Stanley Burg almost a quarter of a century ago) [46, 47]. In the Burg system, produce is stored under atmospheric pressure in the range of about 1–10 kPa at refrigerated temperatures. At this low pressure, a constant circulation of fresh air, substantially saturated with water (RH 80–100%), is maintained. Facilities to constantly scrub CO_2 and ethylene could be included as well. Although the system performed rather well, and shelf lives of different horticultural and floricultural products could be extended 3- to 10-fold, it was technically complex and for this reason was never used as widely as CA or MA storage. Vacuum packaging (VP) may be regarded as a special type of MAP, since part of the normal headspace is removed, leaving an altered initial atmosphere that is not controlled after packaging. VP puts quite a pressure strain on produce and is only suitable when the product is sufficiently durable.

Using a VP system—called a moderate VP system because it operates at 40 kPa—a significant prolongation of quality shelf life at 8°C was obtained with a range of minimally processed fruits and vegetables [48]. In this system, the initial gas composition is that of normal air, but because of the reduced partial gas pressure, the amount of O_2 available at the start of storage is about one-third of the normal amount. As with MAP, the lower O_2 content stabilizes the postharvest product quality by slowing the metabolism of the produce and the growth of spoilage microorganisms. Compared to refrigeration-only storage, refrigerated storage under moderate vacuum was found to improve microbial quality (e.g., red bell pepper, chicory endive, sliced apple, sliced tomato), sensory quality (e.g., apricot, cucumber) or both (e.g., mung bean sprouts and a mixture of cut vegetables). In some instances no beneficial effect (mushroom, green bell pepper, and a mixture of cut fruits) or an impeded decrease in sensory quality (strawberries, alfalfa) was noticed. With cut products (vegetables and fruits salad mixes, chicory endive, apple), VP strongly retarded enzymatic browning of the cut surfaces.

22.4.5 Modified-Humidity Packaging

MAP, CAP, and VP all focus on changing the metabolic gases oxygen and carbon dioxide. Modified-humidity packaging (MHP), however, is designed for products where dehydration causes the most important quality losses, and therefore focuses on controlling water vapor levels. When products such as leafy vegetables or bell peppers are not packed, very soon quality losses can be observed (e.g., wilting and shriveling). In most "closed" packages such as MAP, CAP, and VP, the relative humidity is close to saturation due to the water exchange between the product and the headspace. This high humidity increases the probability of condensation and free water accumulating directly on the product, especially when the package is exposed to changing temperatures. Therefore, MHP systems are designed to control not only dehydration but also condensation. The in-pack relative humidity (RH) is influenced by the rate of water loss (transpiration) of the product and the transmission rate for water vapor of the package, which are dependent on the prevailing water vapor pressure and temperature of storage. Temperature is one of the most important factors determining the in-pack RH. Weight loss relates more exactly to the vapor pressure deficit than to relative humidity, but at constant temperature, weight loss has a linear relationship with relative humidities above 75–85% [49]. At higher temperatures the air can contain more water vapor, thereby decreasing the RH value. A package designed to have a high relative humidity at a high temperature will show condensation on the package surface or on the product if the temperature is decreased substantially. To counteract the effect of condensation, films have been developed that are coated with an antifog layer, due to which moisture forms a continuous layer rather than separate droplets on the surface

of a film. This allows a clear view of the product and prevents water from forming a pool at the bottom of the package.

For many products, transpiration must be reduced in order to maintain quality. Products with a large surface area such as lettuce and endive are very susceptible to wilting. Bell peppers and tomatoes also benefit from good control of relative humidity [50, 51]. Reducing water loss is one of the main aspects related to packaging of minimally processed products, despite the usual emphasis on gas levels [2]. On the other hand, for products such as onions and flower bulbs, humidity should not be too high, as it results in increased sprouting. Like O_2 and CO_2, water vapor levels can be too high or too low, and an optimum level should be reached. For (Israeli) bell peppers this level was estimated to be 92% relative humidity at 8°C [52]. A lower relative humidity caused too much weight loss, while a higher relative humidity caused decay. Especially for products where water loss is the predominant cause of quality changes (e.g., bell pepper and tomato), MHP can be effectively used to minimize loss of quality. In such cases, the concentrations of oxygen and carbon dioxide in MHP are often close to that of ambient air.

Many commercially available packaging materials that have favorable gas-permeability characteristics for a certain commodity cannot be used because they have a rather low permeability for water vapor. When the in-pack RH is very high (≥95%), a small fluctuation in storage temperature results in condensation, which greatly enhances the proliferation and spread of spoilage microorganisms. Especially for fruits, the high RH conditions cause heavy losses due to microbial decay. Control of the in-package RH may be pursued through the use of packaging materials with high water vapor permeabilities; by inclusion of sachets containing water absorbers like $CaC1_2$, sorbitol, or xylitol in the package ("active packaging"); or by use of packaging materials with suitable gas permeabilities onto which such desiccants are coated [51, 53].

22.5 IMPORTANT PARAMETERS IN PACKAGE DESIGN

22.5.1 Product Characteristics

Before a package can be designed, detailed knowledge about the physiological characteristics of the product to be packed and the environmental conditions the package is exposed to after production is essential. Many specific parameters need to be known. Not only are optimal O_2, CO_2, and water vapor levels important, but so too are the upper and lower limits of these components beyond which damage can be expected. When low O_2 and/or high CO_2 is beneficial, it becomes important to quantify the relationship between gas conditions and gas-exchange rates. For good quantification, O_2 uptake and CO_2 production should be measured under a range of O_2 and CO_2 concentrations. Such data sets, however, are still scarce.

Another important product aspect is the influence of light on color changes, for instance, with chicory endive, which changes from the preferred yellow-white to the undesired green color under excess illumination. It is also important to know what mechanical properties the package should have when delicate products such as berries are to be packaged without mechanical damage.

22.5.2 Package Characteristics

An important aspect of package design is the selection of the packaging material, and this can be a cumbersome exercise. Exama et al. [54] studied the possible application of 20 different types of polymer films and was still not able to find a suitable match for products with a high respiration rate. Using too high barrier package film, O_2 will be fully depleted and fermentation will lead to off-odors and off-flavors. In addition, the right combination of low O_2 and high CO_2 is crucial. This highlights two decisive aspects in selecting films: (i) the permeability for O_2 and CO_2 at the temperature to be used, and (ii) the ratio between O_2 and CO_2 permeability. A serious drawback is that gas permeability specifications given by film manufacturers are usually determined under conditions remote from the high-humidity refrigerated storage conditions of respiring produce. Thus, it is impossible to deduce only from the specifications provided by film manufacturers whether a specific film would provide for an in-package gas atmosphere with tolerable O_2 and CO_2 levels when applied in practice. Thus, the suitability of a film must be tested with the product under the correct practical conditions.

In addition to the permeability of the metabolic gases, permeability for water vapor, ethylene, and volatiles can be important. A low permeability for water vapor can increase the risk of condensation. Condensation should always be avoided since it generates an ideal climate for microbial growth. Also, discoloration of the product can result from condensation.

Currently, polyethylene (PE) and polyvinyl chloride (PVC) films are the most often used polymers. In the past decade, a new type of film was introduced, with very small holes (microperforation) as the main pathway for diffusion [55]. The interesting aspect of these films is that diffusion of O_2 and CO_2 through the film is equal. This enables the creation of packages with both low O_2 and high CO_2 concentrations. Such atmospheres are especially suitable for minimally processed products but also for unprocessed products with extremely high respiration rates like asparagus, broccoli, mushrooms, or mung bean sprouts.

In addition to the selection of the type of film, other important aspects include the thickness of the film, the surface area used, the package volume, and for films with microperforation, the number of holes per area. Film thickness, film area, and the number of holes influence the equilibrium gas composition inside the package. Varying package volume and the free volume inside the package influences the rate at which gas concentrations are changing. The final equilibrium concentrations will be equal, but the moment in time at which these concentrations are reached can differ by varying the volume.

22.5.3 Modeling

Since there are so many variables to take into account in package design, a trial-and-error type of approach can lead to numerous

attempts to find the best package. The risk is that the best package will not be found. Sufficient control of the many different factors interacting in determining the atmosphere change in a MAP can only be achieved with the help of mathematical modeling [56]. Mathematical models may provide a means to determine and predict important packaging specifications. When optimal (equilibrium) gas conditions are known as well as the respiratory response to various O_2 and CO_2 concentrations, the suitable permeability characteristics of the package can be mathematically deduced. The most frequently used models that relate gas conditions to O_2 uptake and CO_2 production are based on Michaelis–Menten kinetics [2, 14, 57]. Although inhibition of CO_2 on respiration is not found for all products, Peppelenbos and van 't Leven [58] examined which type of inhibition best described the influence of CO_2. The models of Banks et al. [14] or Peppelenbos et al. [59] can be applied best when not only respiratory CO_2 but also fermentative CO_2 production needs to be calculated. An example of gas-exchange modeling is given in Figure 22.1, where at low O_2 concentrations CO_2 production increases due to enhanced fermentation.

The description of gas diffusion through packaging materials is mostly done by applying Fick's law [2]. Although all models mentioned earlier are static, they can be incorporated into dynamic models to be used for the prediction of changing gas conditions inside a package [60]. Packages can be easily designed with such dynamic models by changing variables such as film type, surface area, and amount of product packed. A very useful extension of simulation models would be the incorporation of the expected variance of the achieved equilibrium conditions. Using expected variance, not only can optimal packages be designed, but so can suboptimal packages that are also safe. A survey of this variance has already been carried out for broccoli by Talasila et al. [61].

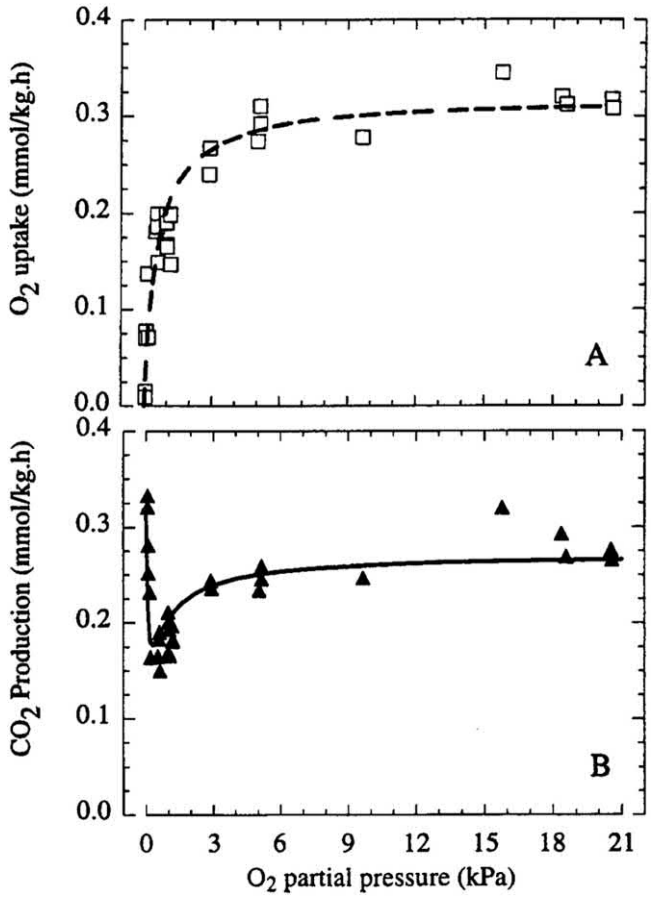

FIGURE 22.1 Gas exchange rates of strawberries (*Fragaria ananassa* cv. *Elsanta*) at 4°C. (A) O_2 uptake rates (□) with O_2 uptake model. (B) CO_2 production rates (▲) with CO_2 production model. (From Peppelenbos et al. [59].)

Since temperatures in the distribution chain often cannot be strictly controlled, another interesting feature of dynamic modeling is the possibility of simulating products passing through the different links of the distribution chain. Using simulation, for instance, the dynamics of the gas composition inside the package can be evaluated in order to determine whether gas conditions will remain within the limits of tolerance of the commodity. When necessary, the use of different packaging films can be simulated in order to obtain the most optimal equilibrium modified atmosphere condition. The end result of the modeling exercise, however, could be that there is no suitable packaging film available commercially that would be suitable for use. Instead of not packing the product, this information could be used to further improve distribution chains or to give suggestions to the packaging industry, defining the requirements for new films in terms of their temperature sensitivity, CO_2:O_2 permeability ratio, etc.

Once proper models have been created and integrated in package design, they should be mandatory in the development of packages that achieve optimal gas conditions at dynamic temperatures encountered in practical situations and/or of packages that can overcome fluctuations in temperature, which temporarily cause gas conditions to exceed tolerance limits but do not affect product quality.

22.6 MICROBIAL GROWTH UNDER MODIFIED ATMOSPHERES

22.6.1 Spoilage Microorganisms

Fresh fruits and vegetables normally have an elaborate spoilage microflora, due to intensive contact with various types of microorganisms during growth and postharvest handling. The high acidity of many fruits (pH <4.6) limits spoilage to acid-tolerant molds, yeasts, and lactic acid bacteria. Vegetables generally have a pH of around 6.0–7.0 and lack this intrinsic protection. Microbial spoilage of undamaged, healthy products can only be effectuated by microorganisms able to penetrate through the skin, which requires the presence of specific enzyme systems. In vegetables, pectinolytic gram-negative bacteria of the genera *Pseudomonas* and *Enterobacter* are often involved in spoilage. The effect of MAP, and in particular carbon dioxide, on spoilage organisms is distinctly selective, but it is possible to make some broad generalizations. Molds exhibit sensitivity, while yeasts are comparatively resistant. Different species of bacteria, on the other hand, vary greatly in sensitivity. For example, aerobic organisms such as

Pseudomonas, *Micrococcus*, and *Bacillus* are inhibited by CO_2, while the *Lactobacillus* species are more resistant. On the other hand, facultative anaerobes such as *E. coli* are less affected by the level of CO_2 but more by the level of O_2. Most spoilage organisms that pose a quality problem in produce are aerobic, thus a limited supply of O_2 hampers their growth potential. Nitrogen has little inhibitory effect except in displacing oxygen.

Spoilage microorganisms usually pose no safety problem for consumers. The main concern is that applications of modified atmospheres diminish the competition for oxygen, carbohydrates, other nutrients, and space between spoilage microorganisms and pathogens. This may allow the growth of certain pathogens to hazardous levels, especially during extended shelf life. In addition, the problem of product temperature abuse, either during manufacture, distribution, and retail or by the consumer, must also be considered. In those cases spoilage microorganisms may be important safety indicators, giving an organoleptic warning signal to consumers that the food product has been mishandled or kept beyond its shelf life and therefore may not be safe to eat. However, when technologies such as MAP are used to extend the product shelf life by suppressing spoilage organisms but not all hazardous pathogens, situations could occur in which the packaged food is organoleptically unspoiled but very unsafe to eat.

22.6.2 Pathogenic Microorganisms

Most fruit products have too low a pH to permit the growth of pathogenic bacteria—only figs, peaches, and tomatoes, whose pH is potentially in the range 4.6–4.8, may permit pathogenic growth. In the early days of MAP, attention was focused primarily on anaerobic pathogens, especially proteolytic *Clostridium botulinum,* which produces a deadly toxin but does not grow below 10°C. Since nearly all MAP foods are refrigerated, focus has been mainly on the survival and outgrowth of cold-tolerant pathogens such as *Yersinia enterocolitica* and *Listeria monocytogenes* that can proliferate under low-oxygen conditions [6, 39]. An important factor with respect to microbial safety is whether the MAP food is intended for direct consumption or requires heating before consumption. With MAP foods that are cooked before being eaten, vegetative pathogens should all be killed, provided the cooking instructions are properly followed. However, the majority of MAP produce is sold as "ready-to-eat." The main potential sources of pathogenic bacteria in fresh and minimally processed produce are the raw material, ingredients, plant workers, as well as the processing equipment and environment. In the following, the main pathogens of possible concern to MAP are described.

22.6.2.1 *Clostridium botulinum*

Because of the potency of their toxin, the potential growth of *Clostridium* species in MAP foods has been of especially great concern [62]. The organisms can be present in soils and can thus come into contact with fruits and vegetables easily. *C. botulinum* is not markedly affected by the presence of CO_2, and growth is encouraged by the anaerobic conditions that may exist in MAP. Most strains of *C. botulinum* do not grow at temperatures below 10°C, although nonproteolytic *C. botulinum* types B, E, and F have been recorded as growing and producing toxins at temperatures as low as 3.3°C. Botulism has been linked to coleslaw prepared from MA-packaged, shredded cabbage mixed with coleslaw dressing [63, 64]. Shredded cabbage onto which spores of *C. botulinum* types A and B were inoculated and that subsequently was MA packaged and held at room temperature was found organoleptically acceptable after 6 days, yet type A toxin production was apparent on day 4. A pungent odor was produced and released on opening the bag, after which the cabbage smelt normal. A recent survey by Lilly et al. [65] on the incidence of *C. botulinum* in MAP and VP vegetables involving 1118 packages of a variety of precut produce (including cabbage, pepper, coleslaw, carrot, onion, broccoli, mixed vegetables, stir-fry vegetables, and various salad mixes) found that 1 package each of shredded cabbage, chopped green pepper, and Italian salad mix contained *C. botulinum* type A spores, while an additional salad mix (main ingredient, escarole) contained both *C. botulinum* type A and type B spores. The overall incidence rate (0.36%) of *C. botulinum* spores thus may be quite low in commercially available precut vegetables.

22.6.2.2 *Listeria monocytogenes*

The widespread presence of this organism in the environment and its ability to grow at low temperatures makes it a pathogen of special concern. A serious outbreak of listeriosis was thought to be derived from cabbage fertilized with manure from infected sheep [66]. In many studies of a variety of produce carried out since this outbreak, it was frequently established that *L. monocytogenes,* when the organism was inoculated onto vegetables, grew as well under MA or CA conditions as in air at 4°C or 15°C [37, 41, 67–71]. By now, growth of this pathogen under MA conditions has been reported for asparagus, broccoli, cauliflower, lettuce, and chicory endive. Studies by Carlin et al. [40] investigated the fate of *L. monocytogenes* in minimally processed foods in the presence of nonpathogens and at temperatures ranging from 3°C to 20°C. It was shown that on unspoiled products *L. monocytogenes* would hardly grow more than 2 log units whatever the storage temperature, but that spoilage of the salad leaves would permit a rapid multiplication. Low storage temperatures reduced growth of *L. monocytogenes* more than that of the spoilage microflora and are therefore a factor improving safety. Carbon dioxide concentrations of 10–20% reduce spoilage development and growth of the spoilage microflora, whereas higher concentrations slightly increased growth of *L. monocytogenes.* On minimally processed green endive, it was found that that high inoculum concentrations overestimated the maximum growth of *L. monocytogenes.* Again, the epiphytic microflora of green endive leaves had a barrier effect against *L. monocytogenes.*

22.6.2.3 *Aeromonas hydrophila*

This psychrotrophic organism is also widespread in the environment and is mainly waterborne. It has been found in drinking water, fresh and saline water, and sewage water. Cytotoxic strains have been found on seafood, meats, and poultry, as well as on fresh produce, parsley, spinach, celery, and endive. The pathogen grows rapidly at refrigeration temperature [72]. Berrang et al. [73] observed that *Aeromonas* could grow to population densities exceeding 10^6 CFU/g within 2 weeks at 4°C on asparagus, broccoli, and cauliflower, and that CA conditions did not markedly affect its growth potential.

22.6.2.4 *Yersinia enterocolitica*

Animals, specifically swine, are the predominant natural source of *Yersinia enterocolitica*. However, this cold-tolerant pathogen has also been isolated from raw vegetables. As with the former two pathogens, modified atmospheres optimal for produce do not hamper its growth at refrigeration temperature. With little oxygen (1.5%) present, carbon dioxide levels as high as 50% were found to be required before its growth was significantly reduced [36].

22.6.2.5 *Bacillus cereus*

This bacterium, a common contaminant of vegetables, does not usually grow below 10°C [74]. However, recent reports have shown that some enterotoxigenic strains can grow at temperatures as low as 4°C and produce toxin at 8°C. *B. cereus* is rather susceptible to the antimicrobial effects of CO_2 [36], and CO_2-rich environments severely reduce the ability of the spores to germinate.

22.6.2.6 *Salmonella* spp.

This organism is most commonly associated with animals and birds and is only present on vegetables through cross-contamination. Nevertheless, two large outbreaks of salmonellosis have been attributed to fresh produce, both involving tomatoes stored at ambient temperatures [75, 76]. *Salmonella* species have also been implicated in smaller outbreaks in which raw bean sprouts and different types of melons were the vehicles. Although high levels of CO_2 retard the growth of *Salmonella,* generally the inhibitory effect on the organism is largely dependent on decreased temperature. Most *Salmonella* species are mesophilic bacteria, but many isolates survive well during storage at 5°C [77].

22.6.2.7 *Staphylococcus aureus*

S. aureus does not grow well under chill conditions or in the presence of competing microorganisms. The pathogen has been found on fresh produce and ready-to-eat vegetable salads. It is known to be carried by food handlers. Generally, CO_2 has an adequate inhibitory effect on the growth of *S. aureus* when combined with low-temperature storage.

22.6.2.8 *Escherichia coli*

E. coli is a mesophilic bacteria often used as an indicator of fecal contamination. Enterotoxigenic *E. coli,* the common cause of travelers' diarrhea, is regularly detected on raw vegetables. Strains can grow at temperatures below 10°C, but not usually below 7°C. Some strains reportedly are able to grow and produce toxin at 5°C. The growth of this organism can be inhibited by high levels of CO_2. Enterohemorrhagic *E. coli* O157:H7 is recognized as an important emerging pathogen. Outbreaks of this pathogen have been associated with unpasteurized apple cider and cantaloupe. Also, broccoli is suspected to have carried this type of *E. coli.* MAP had no effect on pathogen growth on shredded lettuce and cucumber in experiments in which storage temperatures were 12°C and higher [78, 79].

22.6.2.9 *Campylobacter jejuni*

This organism is still one of the major causes of bacterial enteritis. Poultry and other foods of animal origin are the main sources. The pathogen has been implicated in diseases caused by consumption of fruits and vegetables. Cross-contamination of fresh produce with *C. jejuni* from poultry meats has been suspected. The pathogen has been found to survive sufficiently on sliced watermelon and papaya to be a risk for consumers. Total absence of oxygen was noted, whereas survival, without growth, was enhanced in an atmosphere of 100% N_2. Optimal growth has been documented to occur under atmospheres of reduced oxygen level and high temperatures (42–45°C). The minimum growth temperature reportedly is 32°C, so the risk with consumption of refrigerated MAP produce should be minimal.

22.6.2.10 Disinfectant Usage

The use of disinfectants to reduce the microbial load of minimally processed fresh salads is permitted and practiced in many countries around the world. It has been found that after disinfection, the surviving spoilage microorganisms have an increased growth rate and rapidly reach the same level found on nondisinfected products [38, 40]. In the case of contamination with *L. monocytogenes* during processing, disinfection of salad leaves would reduce the antagonism from epiphytic bacteria and might increase the growth of the foodborne pathogen. Therefore, the major role of disinfectants may be to prevent the build-up of contamination in washing water during processing rather than to reduce microbial load of raw salad leaves. In the production of MAP produce, good manufacturing practices should be observed that avoid recontamination of disinfected produce with hazardous pathogens.

22.7 RECOMMENDED MA CONDITIONS FOR PRODUCE

The benefits of CA and MA packaging vary greatly according to the plant product. Storing some products, like apples, under low O_2 conditions can increase the storage period by months. Also some products, like carrots [80], do not respond positively to low O_2 or high CO_2 concentrations. Generally, altered gas conditions are regarded as positive only within a certain range of concentrations, so-called optimum concentrations.

Much research effort has been devoted to the determination of the optimum gas concentrations for individual products [81–83]. Table 22.1 gives a recent update on recommended storage conditions for a range of fruits and vegetables. Traditionally, lists of recommended storage conditions have been developed by national research organizations conducting extensive laboratory research [8]. A common experimental procedure is to store products under a range of O_2 and CO_2 concentrations and to monitor quality changes. The lower O_2 limit for stored fruits is accomplished empirically by lowering the storage O_2 concentration until intolerable damage occurs. Each commodity and new cultivar requires a large investment in time, equipment, and materials [42, 84]. By repeating the trials year after year, it is possible to sense the importance of climatic variation on product behavior [8].

Some caution is needed in the application of optimal concentrations. For the various apple cultivars, for instance, the advised optima differ according to country [82]. Growing conditions like climate and orchard factors influence crop growth and contribute to these differences [85–87]. Although this is probably also the case for other plant products, this is never specified.

Another important aspect of optimal values for temperature, and O_2 and CO_2 concentrations is that they are often established separately, although interactions between temperature, and O_2 and CO_2 concentrations (and probably also humidity) are known. Optimal O_2 concentrations are found to shift to a higher value when CO_2 concentrations are increased [5, 88–90], when products are more mature [5, 91–93], when they are kept at a higher temperature [17, 90, 94, 95], or when products sensitive to chilling injury are stored at too low temperatures [89]. For apples, the CO_2 limit at low O_2 concentrations decreases when the temperature is decreased [10]. The effect of relative humidity is often an interacting factor as a cause for, or in the symptom expression of, a disorder. Relative humidity, however, is often not mentioned and frequently not (accurately) measured [43]. The conclusion is that it is very hard to recommend absolute values for optimum O_2 and CO_2 concentrations or O_2 and CO_2 limits for a product without knowledge of other factors. The understanding of actual physiological processes determining the potential of products for MA is developing steadily and will help the further development of optimal and safe packaging techniques.

TABLE 22.1
Recommended Optimal Storage Conditions

Commodity	Temperature (°C)	RH (%)	$[O_2]$ %	$[CO_2]$ %	Potential
		Fruits			
Apples	1–4	90–95	1–3	0–6	A
Avocado	5–13	90	2–5	3–10	B
Banana	12–14	85–95	2–3	8	A
Blackberry	0–2	90–95	5–10	15–20	A
Blueberry	0–2	90–95	2–5	12–20	A
Cherry	0–2	85–90	3–10	10–15	B
Kiwi	0–2	85–90	1–2	3–5	A
Mango	10–15	90	3–7	5–8	B
Melon	8–10	85–90	3–5	5–15	B
Nectarine	0–2	85–90	1–2	3–5	B
Peach	0–2	85–90	1–2	3–5	B
Pear	0–1	90–95	2–3	0–2	A
Persimmon	0–5	90	3–5	5–8	B
Plum	0–2	85–90	1–2	0–5	B
Raspberry	0–2	85–90	5–10	15–20	A
Red currant	0–2	90	5–10	15–20	A
Strawberry	0–2	90	5–10	12–20	A
Sweet corn	0–2	90	2–4	5–10	B
Tomato	1–13	90	3–5	2–4	B
		Vegetables			
Artichoke	0–2	90	3–5	0–2	B
Asparagus (green)	0–2	95	10–15	7–12	A
Broccoli	0–1	90–95	2–3	8–12	A
Brussels sprouts	0–1	90–95	2–4	4–6	A
Cabbage	0–1	95	2–3	3–6	A
Celery (stem)	0–2	90–95	3–5	1–4	B
Chicory (witloof)	0–2	90–95	2–3	5–10	A
Leek	0–2	90–95	3–5	3–6	B
Lettuce	0–2	90–95	2–3	2–5	B
Mung bean	0–2	90–95	1–2	1–3	A
Onion	0–2	70–80	1–4	2–5	B
Spinach	0–2	95	21	10–20	B

Source: Adapted from Kader [12].

Note: Value based on the proceedings of the 6th and 7th International Controlled Atmosphere Research Conferences (Ithaca, New York, 1993; and Davis, California, 1997, respectively). Potential: A = excellent, B = fair. Products with a low potential or no potential are not listed.

22.8 FUTURE OUTLOOK

Fruit and vegetable consumers increasingly demand high-quality products. An important quality feature is freshness. No signs of senescence, decay, wilting, or shriveling are accepted. In general, consumers are willing to pay more for products with better quality. Often consumers also have specific expectations as to ripening stage. For instance, consumers in Northern Europe want firm, not mealy tomatoes (i.e., the fruits should not be too ripe) but also tomatoes with taste and flavor (i.e., the fruits should not be harvested in a very unripe stage). These quality demands result in strict criteria for storage conditions, transport conditions, and shelf conditions. MA packaging is a tool of increasing importance in meeting these criteria.

MA packages are increasingly being used. The total European MAP market handles on average 300 million package units of produce per year with a market value of about $1 billion [96, 97]. In the United States, a market share of about $8 billion in MAP products has been predicted for the year 2000. Often, successful applications are due to good control of the whole distribution chain, from the moment of packing the product until it is displayed on the retail shelf. When film

permeabilities or respiration rates are not well characterized, package designers have to resort to empirical studies with MA packages that can be described as "pack and pray" [2]. A thorough understanding of principles and processes will lead to a more rational selection of packaging materials [5]. Nevertheless, even a package that has been well designed in a laboratory may not necessarily perform well in practice, when no information on the actual storage, transport, or shelf-life conditions was considered at the design stage.

Current state-of-the-art MAP systems for minimally processed produce have been optimized mainly for product quality. Safety and cost aspects have not yet been optimized. Also, quality deterioration still occurs, and further improvements can still be achieved through, for example:

- A systematic approach to select appropriate gas conditions in MAP for specific products
- Availability of more data on the interaction between produce and gas composition
- Development of better computer software to aid in the selection of suitable packaging systems (gas compositions plus foils) for use under dynamic conditions (temperature, humidity)
- Combating microbial hazards in MAP systems (i.e., psychrotrophic pathogens) using improved MAP systems or new hurdles to microbial growth
- Use of "smart" films that compensate for temperature fluctuations by changing permeability properties
- Studies on more environmentally friendly MAP systems (e.g., simple foils, biodegradable foils)
- Minimizing packaging in MAP systems (including biocoatings as part of the packaging concept)

Table 22.2 lists these and other trends foreseen in modified-atmosphere packaging. A misconception of MAP is that it can overcome hygienic abuses in the production or handling of a product. MAP is not a panacea for the preservation of food products, but, if used correctly, it slows the natural deterioration of a product. There is no enhancement of product quality but, when starting with a good clean product, the initial fresh state of the product may be prolonged. Strict codes of practice should be enforced to ensure the maximum quality shelf life and safety of MA-packed foods.

With the increasing importance of MA worldwide, the role of temperature and of its control in the successful use of MAP should be considered. MA packages will not be a substitute for adequate temperature management. For all nontropical products, only cooling is always better than only MA. However, when only MA can be applied in cases where cooling is not possible, stable temperatures are necessary since no films are yet available that respond to temperature fluctuations of the packed product [54].

Modern consumer demands for convenient and fresh, wholesome produce together with the recent reorganization of the distribution chain will further stimulate the use and the broadening of the application area of MAP. Continued basic physiological and microbiological research on the action of MAs will minimize the risks of loss and safety associated with the use of MAs and will allow for faster MAP optimization using models. New technological developments, especially in the area of more suitable packaging films, will contribute to the success of MA as a preservation technique. The main limiting factors for further expansion of MAP may arise out of environmental concerns. A solution for the huge waste problem, in the long run, may be found in the use of edible and biodegradable films that are able to create a modified atmosphere [98]. The integration of different preservative hurdles, such as refrigeration, MAP, active components, and/or green chemicals in accordance with the concept of combined processing [99], may not only minimize potential microbial problems but also contribute to optimized product quality. The range of powerful technologies we have at our disposal today will help to ensure a good supply of minimally processed, fresh, safe, and ready-to-eat products.

TABLE 22.2
New Developments in Modified-Atmosphere Packaging

Packaging	Active Packaging
Tailoring gas transmission/selectivity (new plastics, microperforation)	Absorbers (O_2, ethylene, water, off-flavors)
Water vapor transmission/selectivity (modified humidity storage)	Generators (CO_2, antifungals, flavors)
Biodegradability—environment (multi/mixed layered plastics fortified with biodegradable mass)	Controlled release (antimicrobials, antioxidants)
Composites (metals/cartons with plastics/liners)	Dynamic packaging
New concepts (high oxygen, noble gases, optimization)	Temperature dynamic films
	Humidity dynamic films
Chain optimization (controlling basics: chilling, handling, logistics)	Biopackaging
	Biodegradable/edible films
	Custom-made physical properties
Minimal packaging	Biocoatings
Integrating functionalities	Edible, physical protection (invisible)
Simpler/less films, better recyclable films	Functional features (antimicrobials)

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23 Glass Transition Concepts in Food Preservation

Mohammad Shafiur Rahman

CONTENTS

23.1 INTRODUCTION

Mechanical, physical, chemical, and microbial effects are the leading causes of food deterioration and spoilage. Damage can start at the initial point by mishandling of foods during harvesting, processing, and distribution; this may lead to ultimate reduction of shelf life. Factors of food spoilage can be extrinsic (environment) and intrinsic (i.e., food compositions and structure). During storage and distribution, foods are exposed to a wide range of environmental conditions. Environmental factors such as pressure, temperature, humidity, oxygen, and light can trigger several reactions that may lead to food degradation. As a consequence of inappropriate use of these factors, foods are altered to such an extent that these rejected or significantly reduced the quality.

In the literature, new concepts and hypotheses are being developed and proposed in the areas of food properties in order to bring food science from empiricism to the strong scientific foundation [1–4]. The stability of foods is of utmost interest to both food scientists and engineers, and a better understanding of the factors controlling stability or reaction rates is clearly needed [5, 6]. In the 1800s, Nicolas Appert gave his method of food preservation by heating foods (i.e., in boiling water) in airtight bottles without understanding of bacterial spoilage. A

theoretical understanding of the benefits of heating (i.e., canning) did not come until Louis Pasteur observed the relationship between microorganisms and food spoilage. The safety criteria of food preservation in canning (i.e., D-value, z-value and F-value), drying (i.e., water activity and glass transition), freezing (i.e., critical temperature and glass transition) and chilling (i.e., critical temperature), and acidic medium (i.e., critical limit of pH) were developed. Water activity and glass transition concepts are the two most successful theoretical foundations developed for determining food stability during processing and storage. This chapter presents the glass transition concept and food stability by explaining its dynamics, theoretical understanding, and applications.

23.1.1 Background

In the middle of the 20th century, scientists began to discover the existence of a relationship between the water contained in a food and its relative tendency to spoil [7]. In the 1980s, Labuza and his group generated significant data on food stability as a function of water activity. They also began to realize that the active water could be much more important to the stability of a food than the total amount of water present [7]. Thus, it is possible to develop generalized rules or limits for the stability of foods using water activity. For example, there is a critical water activity below which no microorganisms can grow. For most foods, this critical range is in the 0.6–0.7 values of water activity. Pathogenic bacteria cannot grow below a water activity of 0.85–0.86, whereas yeasts and molds are more tolerant of a reduced water activity of 0.80, but usually no growth occurs below a water activity of about 0.62. A food product is most stable at its monolayer moisture content, which varies with the chemical composition and structure. This was the main reason why food scientists started to emphasize water activity rather than total water content. Since then, the scientific community has explored the great significance of water activity in determining the physical characteristics, processes, shelf life, and sensory properties of foods. It is now used to predict the endpoint of drying, process design and control, ingredient selection, product stability, and packaging selection.

Recently, the limitations of water activity have been pointed out and alternatives proposed [3, 4]. Water activity is defined at equilibrium, whereas foods with low and intermediate water content may not be in a state of equilibrium and be time–temperature–moisture dependent. The critical limits of water activity may also be shifted to higher or lower levels by other factors, such as pH, salt, antimicrobial agents, heat treatment, and temperature to some extent [4]. The third limitation is the specific solute effects [8], which demonstrated that minimum water activity for the growth of microbial organisms was dependent on the solutes employed to adjust the water activity of a medium [7]. It was observed later that some solutes were more inhibiting. Thus, the water activity of a medium is not the only determining factor regulating microbial response. The nature of the solute used also plays an important role, thus the concept of generalization with water activity is questioned. Moreover, water activity does not provide any indication of the mobility of water and its binding nature to the substrate [9]. In addition, many physical characteristics, such as crystallization, caking, stickiness, gelatinization, collapse, molecular mobility, and diffusivity could not be completely explained based on the water activity concept alone. In this case, the glass transition concept was put forward.

23.1.2 Glass Transition Concept and Food Stability

Glassy materials have been known for centuries, but it is only in the last 70 years or so that scientific understanding of these systems has evolved [10]. Early attempts to describe vitrification concluded that glass is a liquid that has lost its ability to flow, thus instead of taking the shape of its container, glass itself can serve as the container for liquids. Food materials are in an amorphous or noncrystalline state below the glass transition temperature and are rigid and brittle. Glass is not crystalline with a regular structure, but retains the disorder of the liquid state. Physically it is a solid, but thermodynamically it is a liquid. Molecular mobility increases 100-fold above glass transition. In kinetic terms, Angell [11] described a glass as any liquid or supercooled liquid whose viscosity is between 10^{12} and 10^{13} Pa s thus effectively behaving like a solid, which is able to support its own weight against flow due to gravity. To put this viscosity into context, a supercooled liquid with a viscosity of 10^{14} Pa s would flow 10^{-14} m/s in the glassy state compared to the flow rate of a typical liquid in the order of 10 m/s. In other words, a glass is a liquid that flows about 30 μm in a century [12].

The early papers on glass transition in food and biological systems appeared in the literature in the 1960s [13–15]. White and Cakebread [15] first highlighted the importance of the glassy state of foods in determining its stability. They were perhaps the first food scientists to discuss the importance of the glassy and rubbery states in relation to the quality control (i.e., collapse) of a number of high-solids systems. The significant applications of the glass transition concept in the 1980s emerged in food processing when Levine and Slade [16, 17] identified its major merits. Other groups around the globe generated significant data on the glass transition and components of state diagrams for a number of food components.

It has been mentioned in the literature that foods can be considered very stable at the glassy state, since below glass temperature compounds involved in the deterioration reactions take many months or even years to diffuse over molecular distances and approach each other to react [18]. The hypothesis has recently been stated that this transition greatly influences food stability, as the water in the concentrated phase becomes kinetically immobilized and therefore does not support or participate in reactions. Formation of a glassy state results in a significant arrest of translational molecular motion, and chemical reactions become very slow [19]. The rules of the glass transition concept are (i) food is most stable at and below its glass transition (i.e., T_g for samples containing unfreezable water, T_g' or T_g''' for samples containing freezable water), and (ii) the higher the T-T_g or T/T_g or T/T_g' or T/T_g''' (i.e., above glass transition), the higher the deterioration or reaction

rates. Similarly, mechanical and transport properties of biological materials could be related to their glass transition. It is very interesting to see that this concept has been widely tested in foods. In many instances the glass transition concept does not work alone, thus it is now being recommended to use both the water activity and glass transition concepts in assessing process ability, deterioration, food stability, and shelf-life predictions [20].

23.1.3 Dynamics of Glassy State

Phase transitions in foods can be divided into two groups: first-order and second-order. At first-order transition temperature, the physical state of a material changes isothermally from one state to another (e.g., solid to liquid, liquid to gas) by release or absorption of latent heat (e.g., melting, crystallization, condensation, evaporation). Second-order transition occurs (e.g., amorphous state to glassy state) without release or absorption of latent heat [19]. Glass transition is a second-order time–temperature-dependent transition, which is characterized by a discontinuity (i.e., shift) or change in slope in physical, mechanical, electrical, thermal, and other properties of a material when plotted as a function of temperature [19]. The process is considered as second-order thermodynamic transition in which the material undergoes a change in state but not in phase. It is more meaningful to define the nature second-order change in the properties because each measurement technique is based on monitoring change in a specific property, and because a change or break in properties is achieved within a certain temperature range rather than a specific temperature. A perfect second-order transition occurs at a specific temperature.

Glasses are formed when a liquid or a rubbery system is cooled so rapidly that there is no time for the molecules to rearrange themselves and pack into crystalline domains [21]. With continued cooling, the system shows a significant change in thermal, mechanical, and other physical properties at the glass transition region. Experimentally this is supported by calorimetric studies on supercooled glycerol, which produces a step change in heat capacity as a function of temperature at 190 K [22]. During heating, devitrification of polydisperse food materials does not occur at a fixed point with the change of specific heat. Instead, networks soften over quite a large range of temperatures [23]. Researchers may prefer to refer to the molecular processes as glass transition rather than as second-order transition in order to avoid implying a thermodynamic state at which equilibrium conditions are achieved. This is due to the increasing rates of cooling, which shift the glass transition at higher temperatures and produce a less dense glass, arguing that equilibrium glass conditions lie below the experimentally accessible values [24].

23.1.4 Equilibrium and Nonequilibrium State

Complex foods exist in states of either unstable nonequilibrium or metastable equilibrium, but never in true thermodynamic equilibrium [25]. Fennema et al. [25] defined the terminology as follows. Any food consisting of only one phase requires minimization of free energy to attain thermodynamic equilibrium. For foods containing two or more phases, thermodynamic equilibrium requires that the chemical potential be equal, in every part of the system, for each substance present. Chemical potential determines whether a substance undergoes a chemical reaction or diffuses from one part of a system to another.

An *equilibrium state* can be attained through many possible paths, i.e., the same properties must be obtainable at a given temperature regardless of whether the temperature is approached by cooling or warming. Metastable equilibrium refers to a state of pseudoequilibrium, or apparent equilibrium, which is stable over practical periods but is not the most stable state possible. A *metastable state* can exist (i.e., conversion to a more stable equilibrium state does not occur) when the activation energy for conversion to a more stable equilibrium state is so low that the rate of conversion is of no practical importance. *Nonequilibrium* refers to a state that is inherently unstable, i.e., change to a more stable state is likely to occur at a rate of practical importance. The exact rate of destabilization depends on the particular system and the conditions to which it is exposed.

23.1.5 Cooling, Heating, and Different States

The transformation of a material from one state to another depends on heating or cooling as well as their rate and annealing (i.e., history of the sample). Figure 23.1 shows the effect of the heating or cooling rate on the transformation of one state to another state. For example, the fast rate of heating or cooling can transform the material from melt to glass or glass to melt without the transformation of crystalline and rubbery states. In addition, annealing at specific conditions can form the types and characteristics of the states.

23.1.6 State of Water in Foods

Different states of water, such as bound, free, capillary, mobile, nonsolvent, and unfreezable, are defined in the literature [19]. The state of water can be measured with different techniques or methods. The water sorption isotherm is based on the three types of water: monolayer, multiplayer, and mobile or free water [27]. The Brunauer-Emmett-Teller (BET) monolayer is estimated from the water sorption isotherm and commonly presented in the literature. It could be mentioned that only the BET monolayer has strong theoretical basis and should be

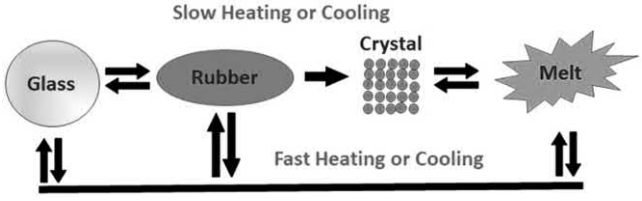

FIGURE 23.1 Effect of cooling rate on the state changes of glass, rubber, crystal, and melt. (Revised from Bhandari and Howes [26].)

used in stability determination. The BET monolayer for large numbers of foods and their components are compiled and presented in the literature. It is not recommended to use the Guggenheim-Anderson-de Boer (GAB) monolayer value due to the number of defects in estimating its real value, although it is popular for its validity up to a water activity of 0.9 [19].

Unfreezable water content can be estimated by comparing differential scanning calorimetry (DSC) endotherms of samples having freezable water. Paakkonen and Plit [28] measured the unfreezable water of cabbage by this method. Unfreezable water can be estimated from the plot of meting enthalpy as a function of water content. This procedure was used for model crackers [29], strawberries [30], dates [31], sucrose [32], and garlic [33]. Usually unfreezable water is independent of total water present in the system. In the case of chitin, unfreezable water increased with the increase of total water, and the amount of freezable water is relatively very low compared to the unfreezable water 106]. In the case of water–gellan systems, unfreezable water increased with the size of the junction zone [34].

Using the nuclear magnetic resonance (NMR) technique, Li et al. [35] studied the mobility of freezable and unfreezable water in waxy corn starch by DSC. Water was found to be isotropically mobile for samples over a range of water contents (6.3–47%) at room temperature. Mobility increased with increasing water content and temperature. A large fraction of unfreezable water was relatively mobile comparable to a liquid state even down to –32°C. The decreasing fraction of mobile water with decreasing temperature suggested that only some of the so-called unfreezable water could be progressively immobilized as temperature decreased. Much of the water remained high in mobility, regardless of the relatively rigid starch molecules in the glassy solid state. This means that water in the glassy state of starch can greatly influence reactions at both ambient and freezing temperatures. At least in this example, the glassy state of the solid materials is not an appropriate term to imply or to predict the molecular dynamics of water and its influence on food stability. Bell et al. [36] determined water mobility in polyvinylpyrrolidone (PVP) systems as determined via NMR and found that water mobility was not affected by the glass transition. PVP systems at constant water activities and water contents, but different physical states (glassy and rubbery), had the same water mobility. An evaluation of four chemical reactions showed no relation between water mobility and kinetic data. The effect of water on chemical reactions is multidimensional and cannot be reduced to a single physicochemical parameter.

It is always found that BET monolayer values are much lower than the unfreezable water [37]. Other techniques used to determine the state of water are dielectric spectroscopy, Fourier transformation infrared spectroscopy (FTIR), x-ray scattering, nuclear magnetic resonance (NMR), magnetic resonance imaging (MRI), electrical resistance, and self-diffusion [9, 38–40]. Three states of water (polymer, capillary, and free) were identified in whey by NMR [41]. The NMR and dielectric measurements of starch paste showed one type of water whereas agar gels contain two types of water when samples contain less than 55% moisture [42]. Lang and Steinberg [43] studied the types of water in corn starch, sugar, sodium chloride, and a mixture of starch and sugar by NMR. It was found that sucrose is a structure-former, while sodium chloride is a structure-breaker. Three types of water mobility were observed in sucrose solution by NMR techniques [44]. Solute–solvent and solute–solute interactions via hydrogen bonding are suggested as mechanisms to explain the observed decrease in water mobility. Lai et al. [45] studied the water mobility in starch-based food products when fat is replaced by fat mimetic components. The active water in the starch–sucrose system was strongly dependent on sucrose content [46]. Molecular mobility of starch and water in starch–water mixtures were studied with the NMR technique and related with the water sorption isotherm [47]. The effect of bound water on glycinin was studied by FTIR spectra [38]. In addition to the aforementioned techniques, many techniques are being used to determine the mobility and state of water and solutes available for chemical reactions, but their interpretation is far from straightforward [9].

23.1.7 Multiple Glass Transition

α-Relaxations are linked to glass transition, which determines the large-scale mechanical stability of the system. The stability of proteins against denaturation and chemical reactivity is much more determined by β-relaxations, which also drive the diffusion of solvents [48]. In many matrices, multiple glass transitions are observed [48]. In the case of freeze-dried tuna fish at moisture content 4.6 g/100 g sample (i.e., unfreezable water), Rahman et al. [49] observed three glass transitions at different temperatures: first one onset at 28.3°C, second one at 97.6°C, and third one at 148.5°C. However, samples at higher moisture content (i.e., 10.0–20.0 g/100 g sample) showed only two glass transitions: one at low and the other one at a higher temperature. The lower glass transition was unaffected by moisture content, whereas higher temperature glass transitions decreased with increasing moisture content (i.e., plasticized). Similarly, in the case of kingfish muscle, the first glass transition was not affected by moisture [50]. In the case of fish muscle, a single glass transition [51–53] as well as two glass transitions [50] were observed. Other multicomponent food systems also showed multiglass transition (i.e., up to three) [54–56]. In the case of sugar-based foods containing freezable water, two glass transitions are commonly observed, one at low temperature and another just below ice-melting temperature [31]. The glass transition temperature before melting decreased with the increase of water, while the lower one remained nearly constant. In the case of rice, three glass transitions were observed, and the second transition showed a decrease with increasing moisture, whereas the first and third ones remained stable (i.e., not plasticized) [55]. Cao et al. [55] hypothesized that only transition decreasing with increasing moisture (i.e., plasticized) would be considered as glass transition. The multiple glass transition could be explained by the natural heterogeneity of biological materials and inhomogeneity due to molecular incompatibility of the

different compositions [49]. In addition, this could be due to the phase separation into biopolymer-rich and plasticizer-rich regions in the matrix [48].

23.2 THEORETICAL UNDERSTANDING OF GLASSY STATE

The present knowledge about glass transition is essentially phenomenological; significant progress on the theoretical aspects is required [57]. Champion et al. [57] reviewed different proposed theories for understanding the glass transition. They are free volume theory, entropy-controlled cooperative motions, mode-coupling theory, frustration-limited domains, and hierarchical correlated molecular motions. A basic and deeper understanding could determine the real applications of glass transition in food technology.

23.2.1 Free Volume Theory

Among all the theories, the free volume theory and relaxation phenomena are beginning to contribute to explaining some changes in foods below glass temperature. Although there is no fully satisfactory theory of the glass transition in polymers, the free volume approach has been widely employed in a quantitative fashion to interpret glassy phenomena in terms of molecular processes [58]. The useful and simple concept argues that total volume per mole of a material is the sum of the free volume and occupied volume. The latter includes not only the van der Waals radii but also the volume associated with the vibrational motion of atoms. The free volume is therefore an extra volume, which is required for larger-scale vibrational motions than those found between consecutive atoms of the same chain [59]. On the free volume concept, glass transition is defined as that temperature at which free volume collapses sensibly to zero and the mobility is restricted, keeping only movement allowed by the occupied volume [60]. Quantitatively, the Williams-Landel-Ferry (WLF) equation is proposed as [60]

$$\log\left(\frac{\mu}{\mu_g}\right) = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)} \tag{23.1}$$

where T and T_g are the experimental and reference temperature, and C_1 and C_2 are the WLF constants (phenomenological coefficients), which are related to the free volume. Other properties, such as shift factor (a_T), G', G'', and rate constant could also be used instead of μ. The universal values of C_1 and C_2 are –17.4 and 51.6, respectively as given by Williams et al. [61]. Soesanto and Williams [62] found these coefficients valid for the WLF correlation of the viscosity of sugars. Ferry [63] identified that these values fluctuate slightly as a function of material types. The VTF equation is also used and expressed as

$$\mu_g = A\exp\left(\frac{BT_o}{T - T_o}\right) \tag{23.2}$$

where T_o is the reference temperature, and A and B are parameters. Angell et al. [64] and Angell [65] classified material as strong or fragile through the glass transition based on the variations of B and C_2. The fragility parameter, m, was introduced to differentiate fragile systems ($100 < m < 200$), which are highly sensitive to temperature changes above glass transition, from strong ones ($16 < m < 100$), which are less disturbed by passage through the glass transition. The applications of various methods to estimate m for food materials are available in the literature [66, 67]. Champion et al. [57] discussed different values of m for food components and their practical significance of the strong/fragile classification with respect to foods. They mentioned that a small variation in the m value for two products may result in a large difference in stability, as the sensitivities to temperature around glass transition are different. It could also benefit from a better knowledge of the strong/fragile behavior of the material processed, such as extrusion, puffing, or flaking. The theory of free volume has been quite successful in following the temperature dependence of viscoelastic functions in the glass transition region and relating parameters to molecular characteristics. The complex nonlinearity and nonexponential character could not be explained much with free volume approach. The popularity of the WLF/free volume approach is due to its simplicity, and it considers that all relaxation processes have the same temperature dependence. In addition, it does not consider the intermolecular interactions, which are more fundamental and the ultimate determining factors of molecular dynamics in densely packed polymers [68].

23.2.2 Relaxation in the Glassy State

The glass transition temperature is a kinetic and relaxation process associated to the primary relaxation of the material [57]. The relaxation time (characteristic time of mobility) is the time that is necessary for the recovery of equilibrium conditions after perturbation of one property of the material. As a relaxation phenomenon, the glass transition temperature can be studied with techniques such as mechanical or impedance spectroscopies, termed α-relaxation (main relaxation). When the temperature is well above glass transition, the molecules or the structural units (such as the repetitive element of a polymer) can move independently from each other because there is enough free volume between entities. The molecular organization in the material is strongly dependent on the temperature above glass transition, but it is relatively stable below glass: molecules stay in an isoconfigurational state, the cooperativity effect being restricted [57].

Within the glassy state, the change in dynamic properties obeys the Arrhenius law. Relaxation processes can be observed in the glassy state with mechanical or impedance spectroscopies. This is also evident with endothermic features on DSC curves. In addition to the main relaxation α, several secondary relaxation β are observed. Its origin still being investigated and it corresponds to more localized molecular motions (for example, in the case of sugars molecule it is linked to the presence and motions of –OH groups), the

molecular structure of carbohydrates, and the rotation of the whole molecule in heterogeneity of the matrix [69–74]. Below glass, a microstructural change may take place, which corresponds to the system approaching the metastable equilibrium, with some extra loss in enthalpy and volume. This *physical aging* can be regarded as a continuation of the α relaxation. The more compact molecular organization and the strengthening of interactions result in changes in mechanical and in transport properties. Its relevance is being increasingly recognized with cereal products [57, 69].

Physical aging is responsible for the appearance of various features on DSC curves: endothermic overshoot that expresses rapid enthalpy recovery after aging below glass transition, but also an exothermic event when a rapid cooling is followed by a much slower rewarming [67]. Two other characteristics need to be explained in the glass transition domain: nonlinearity, which means that the characteristic is changing with time, as it depends on the structure of the glass; and nonexponential behavior, which means that the process of α-relaxation cannot be described by a single relaxation function, due to microstructural heterogeneities. Nonexponential nature is most commonly interpreted as a distribution of relaxation times and mathematically represented by a so-called stretch exponential function [57].

$$\Phi = \exp[-(\theta / \tau)^k] \tag{23.3}$$

where ϕ is the mechanical property, and exponent k is close to 1 for strong liquids (nearly exponential relaxation). For fragile liquids, it changes from near 1 at high temperature to a value close to 0.3 to 0.5 near glass transition [57]. In this situation, Ngai et al. [68] used the Kohlrausch–Williams–Watts (KWW) stretched exponential function. In the case of the stress relaxation modulus:

$$\Phi = \left(\Phi_g - \Phi_e\right)[\exp(\theta / \tau)^{1-n}] + \Phi_e \tag{23.4}$$

where ϕ_g is the glassy mechanical property, ϕ_e is the equilibrium mechanical property of the local segmental motion, θ is the time after the application of a fixed strain, τ is a measured relaxation time, and n is the coupling constant, which ranges from 0 to 1.0. It was found that strongly coupled (interacting) systems have high values of n and an apparently broad distribution of relaxation times, which is the cornerstone of the coupling theory [75]. The parameter n need not be constant during a relaxation, hence the theory is not thermorheologically simple. Kasapis [23] anticipated that much attention will be focused in the area of coupling theory in the future. Other types of theoretical models are discussed by Champion et al. [57].

23.2.3 Critical Temperature of LF-NMR Relaxation and DSC Glass Transition

In the case of rice, low-field nuclear magnetic resonance (LF-NMR) relaxation showed three types of protons from their relaxation times: strongly bound (T_{2b}), moderately bound (T_{21}), and weakly bound (T_{22}) [76]. The T_{2b} and T_{21} increased with the increase of temperature and reached a plateau followed by a sharp increase with a peak (Figure 23.2). It was observed that the second critical temperatures (i.e., end of plateau or onset of sharp increase) were close to the DSC glass transition [76, 77]. The LF-NMR critical temperature was 5°C lower than the DSC onset glass transition (i.e., non-waxy rice: 28.5°C and waxy rice: 26.3°C, moisture: 10.0 g/100 g sample) [78]. In the case of pomegranate skin powder (moisture: 7.7 g/100 g sample), it was observed that this critical temperature was the same as DSC glass transition (i.e., 20°C) or 9°C higher than modulated DSC (MDSC) glass transition (i.e., 11°C) [79]. Similarly, critical temperature was observed lower than the DSC glass for maltodextrin [80] and freeze-dried γ -globulin formulations [81]. It was pointed out that proton mobility was more sensitive to the protein stability as compared to the DSC glass transition. The lower critical temperature from proton mobility could be explained from the mobility distance scale. The LF-NMR measured lower mobility distance (i.e., only proton mobility), while DSC observed a transition when the main chain or substantial proportion of the matrix showed an increased mobility [77]. In the case of biodegradable polymers, the critical temperature (i.e., 75°C) was 5°C higher than the DSC glass transition (i.e., 70°C) [82].

23.3 APPLICATIONS OF GLASSY STATE IN FOOD SYSTEMS

A low glass transition temperature means that at room/mouth temperature, the food is soft and relatively elastic, and at higher temperatures, it may even flow. In contrast, a food with a high glass transition temperature is hard and brittle at ambient temperature. Glass transition and state diagram concept are widely applied in foods to explore its validity. The following section provides a review of this aspect.

23.3.1 Diffusion Process

Glass transition affects diffusion-controlled chemical reactions through the decrease of the diffusion coefficient [83, 84]. The decrease in diffusivity is due to the changes in viscosity and mobility. The diffusion time of a water molecule over 1 Å distance should be more than 10^6 years at room temperature in a glassy matrix based on the Stokes-Einstein relation [57]. The time scale for the loss of stability in food at low water content is not so large. Ablett et al. [85] measured translational diffusion coefficients of water using the pulsed-field gradient NMR technique in 81% pullulan. They showed that diffusivity of water is around 4×10^{-11} m^2/s at glass transition. There was no important drop below the glass, although there was a change in the slope above or below glass transition when diffusivity was plotted as a function of temperature. The diffusion coefficient of water in low-moisture food polymers decreased with the decrease of moisture content without any break in the glass transition when plotting diffusivity versus

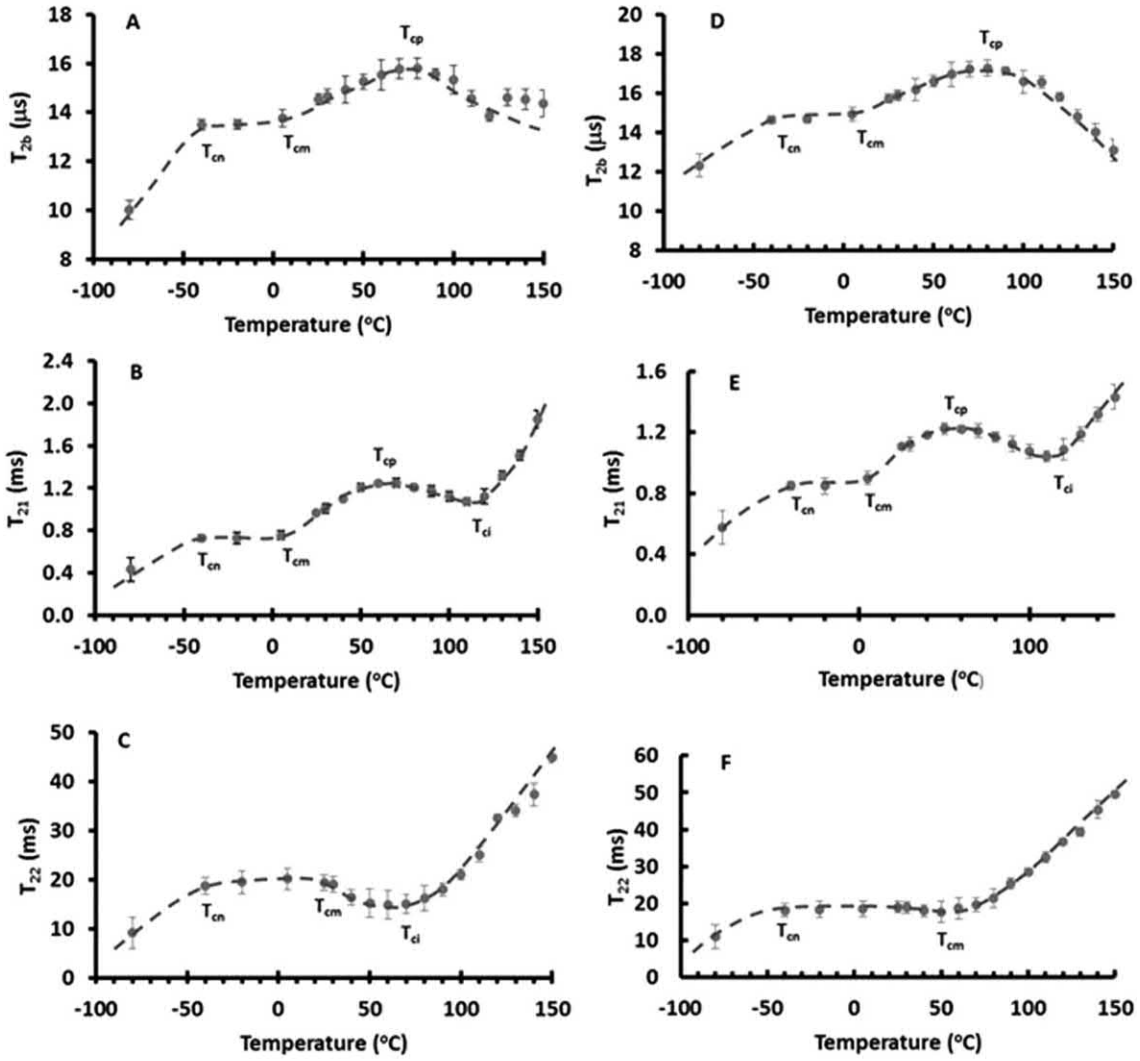

FIGURE 23.2 Relaxation time as a function of temperature. A–C: Nonwaxy rice. D–F: Waxy rice. (From Srikaeoa and Rahman [76].)

water content. The diffusivity depends mainly on moisture content and exhibits a low sensitivity to the nature of surrounding polymer [86]. In case of water diffusivity in maltose–water mixtures above and below glass transition, Parker and Ring [87] plotted diffusivity and viscosity as a function of T_g/T and found that the slopes for diffusivity and viscosity with temperature were not parallel, but instead were extremely divergent when $T_g/T > 0.8$. This decoupling evidence (between viscosity of the diffusion medium and diffusion of small molecule) indicated that the decrease of diffusivity near or below glass is not solely due to the decrease of viscosity. Other important factors that affect the diffusivity in addition to the glassy state are porosity, porous structure, and the structural collapse of foods. The release of entrapped volatiles and flavors are important in food stability. The Arrhenius plot of diffusivity of helium, methanol, and ethyl bromide and glass transition temperature of the sucrose:raffinose matrix showed changes in slope at glass transition [88].

When the molecular size of the diffusing molecule is very small compared to the molecules of the matrix, the Stokes-Einstein relation is inadequate to predict the reactant diffusion. In the case of solute diffusion, macroscopic viscosity did not significantly reduce the diffusion of small molecules [89–91]. The decoupling effect from Stokes-Einstein based on viscosity was also observed for diffusion of fluorescein in sucrose solutions [57]. The decoupling started at $T_g/T > 0.86$ and the discrepancy increases as temperature reached close to glass transition. The motion of small probes in such viscosity materials may be facilitated by the presence of nanodefects and/or by local motions in the material (secondary relaxations).

23.3.2 Texture and Structure

If the moisture content of crispy products, such as chips, crackers, corn flakes, and extruded products, increases due to water sorption or if the temperature is increased during storage, the crispy structure is lost [57]. Roudaut et al. [92] showed that the loss of the crispy texture of dried white bread corresponds to critical water content of 9–10% for which

samples are in the glassy state. A sharp decreasing trend was observed when the compressive failure stress of the frozen sample is plotted as a function of temperature. This change was defined as the brittle–ductile transition. It is not generally true that the brittle–ductile transition appears at the glass transition, although in certain cases the brittle-ductile transition coincided with the glass transition temperature [93]. Nicholls et al. [94] clearly demonstrated that the brittle–ductile transition occurred within gelatinized starch, whereas it was still in the glassy state as defined by the DSC. Watanabe et al. [95] found that there for fish meat. They pointed out that the reasons why these are different needs lengthy and detailed experimentation because the brittle–ductile transition depends on a number of extrinsic factors including strain rate, temperature, stress state, specimen geometry, and the presence of notches and flaws.

23.3.3 Crystallization

Temperatures above glass transition allow molecular mobility and the rearranging of molecules to the crystalline state [16]. Crystallization releases adsorbed water, which in closed containers further plasticizes the remaining amorphous portion of the material [96, 97]. Crystallization causes the most drastic changes to the physical properties of food polymers. It may considerably affect food stability and it may cause impaired rehydration properties of food powders [96–98]. It also affects textural properties, e.g., crystallization of starch in bakery products causing staling [84]. Lactose crystallization in milk powders leads to increased free fat and flavor deterioration, and it may promote nonenzymatic browning [96, 99]. The crystalline materials are not able to entrap other compounds, which become completely released due to crystallization, thus volatiles are lost and lipids become exposed to oxygen.

Increasing $T–T_g$ causes an increasing rate of crystallization with increasing crystallinity. Sandiness in ice cream resulted from lactose crystallization [100]. The crystallization time of amorphous lactose can be predicted using the WLF equation [97]. The extent of crystallization and melting behavior of gelatinized cornstarch was affected by ($T–T_g$) [101]. Crystallization under low $T–T_g$ conditions appears to produce smaller and less perfect crystallites than those produced under high $T–T_g$ conditions, due to lower molecular mobility. The crystallization of isomalt in plasticized form was reduced by adding hydrogenated starch hydrolysates (HSH). The low molecular HSH appeared to be more effective in reducing crystallization than high molecular HSH showing that high glass transition is not necessarily the best inhibitor of isomalt crystallization [102]. In honey stored at 20°C coarse crystals were formed with melting temperatures between 45°C and 65°C, whereas honey stored at –20°C granulated as a finely grained, fondant-like honey, with melting between 25°C and 45°C . In honeys stored at 10°C and 4°C big and small crystals were produced having intermediate characteristics when compared with honeys stored at 20°C and –20°C [103].

23.3.4 Stickiness

Initiation of viscous flow, caking, and stickiness depends on the glass transition temperature. In the case of spray-dried lactose, caking and collapse was increased with the increase of $T–T_g$ [104]. The sticky point of an amorphous sucrose and fructose was found 10°C higher than glass transition [105].

23.3.5 Grain Damage by Drying

It was found that for Bengal variety rice grain at moisture content of 15% (wet basis), the values for thermal expansion coefficients β_{glass} and β_{rubber} were $8.60 \times 10^{-5}°C^{-1}$ and $4.99 \times 10^{-5}°C^{-1}$, respectively [106]. Similarly for Cypress at 14% moisture content, the values for β_{glass} and β_{rubber} were $8.80 \times 10^{-5}°C^{-1}$ and $4.26 \times 10^{-5}°C^{-1}$, respectively. As it can be seen, there is a considerable difference between the values of β_{glass} and β_{rubber} in the two zones on the state diagram. A hypothesis based on glass transitions inside rice kernels was proposed to explain rice fissure formation during the drying process [106–108]. Similarly, when drying occurred in the glassy region, head rice yield was not reduced noticeably after drying. When drying occurred in the rubbery region and no tempering was performed immediately following drying, head rice yield reduction would be marginal if the drying durations were shorter than the maximum moisture content gradient time [109, 110].

23.3.6 Pore Formation in Foods

The glass transition theory is one of the concepts that have been proposed to explain the process of shrinkage, collapse, fissuring, and cracking during drying [111–115]. The hypothesis indicates that significant shrinkage can be noticed during processing only if the temperature of the drying is higher than the glass transition of the material at that particular moisture content [116]. The methods of freeze-drying and hot air drying can be compared based on this theory. In freeze-drying, with the drying temperature below or close to T_g' (maximally freeze-concentrated glass transition temperature, it is independent on solids content) or T_g (glass transition as a function of solids content), the material is in the glassy state. Hence, shrinkage is negligible. As a result, the final product is very porous. With hot air drying, on the other hand, with the drying temperature above T_g' or T_g, the material is in the rubbery state, and substantial shrinkage occurs causing fewer pores.

Karel et al. [88] performed freeze-drying under high vacuum (0.53 Pa) and reduced vacuum conditions (90.6 and 209.3 Pa) to obtain varying initial sample temperatures that were below (–55°C), near (–45°C), and above (–28°C). Collapse was determined by measuring apparent shrinkage before and after freeze-drying of apple, potato, and celery. Samples dried at –55°C showed no shrinkage (more pores), while shrinkage increased with the increase of drying temperature justifying the glass transition concept. Recent experimental results dictate that the concept of glass transition is not valid for freeze-drying of all types of biological materials indicating

the need of the incorporation of other concepts [117], thus a unified approach needs to be used. In the case of freeze-drying, pore formation in food materials showed two distinct trends when shelf temperatures were maintained at a constant level between –45°C to 15°C [117]. The materials in group I (i.e., abalone, potato, and brown date) showed a decreasing trend, whereas group II (i.e., apple and yellow date) showed an increasing trend in pore formation. This may be due to the structural effects of the materials. However, the actual temperature history of the sample passing through freeze-drying was measured. The temperature and moisture history of the sample during freeze-drying could explore fundamental knowledge in explaining the real process of pore formation or collapse.

In many cases during convection air-drying, the observations related to collapse or pore formation are *opposite the glass transition* concept [118–121]. The mechanism proposed for this was the concept of case hardening and internal pressure development [116, 120, 121]. They indicated that at a low drying rate (low temperature), the moisture gradient within the product is small and internal stresses are low and hence the material shrinks down fully onto a solid core, and shrinkage is uniform. At a high drying rate (higher temperature), the surface moisture decreased very fast so that the surface became stiff (i.e., case hardening), limiting subsequent shrinkage, thus increasing pore formation [120]. In the case of case hardening, the permeability and integrity of the crust play a role in maintaining the internal pressure inside the geometric boundary. The internal pressure always tries to puff the product by creating a force to the crust.

The glass transition concept cannot explain the effect of crust and internal pressure. For tuna meat, vacuum-drying produced higher porosity compared to air-drying when both samples were dried at 70°C [122]. The porosity of dehydrated products increased as the vacuum pressure decreased, which means shrinkage can be prevented by controlling pressure [123]. Microwave vacuum drying creates a massive vaporization situation causing puffing [124]. This indicates that in addition to the temperature, environment pressure also affects the pore formation, and this effect cannot be explained by the glass transition concept. Similarly, in the case of extrusion, processing temperatures above 100°C created higher porosity, which is contrary to the glass transition concept [125]. This is due to the rapid vaporization of the water vapor at the exit of the die. After analyzing experimental results from the literature, Rahman [115] identified that the glass transition theory does not hold true for all products or processes. Other concepts, such as surface tension, pore pressure, structure, environment pressure, and mechanisms of moisture transport play important roles in explaining the formation of pores. Rahman [115] hypothesized that capillary force is the main force responsible for collapse, so counterbalancing this force caused formation of pores and lower shrinkage. The counterbalancing forces are due to generation of internal pressure due to vaporization of water or other solvents, variations in moisture transport mechanism, and pressure outside the material. Another factor could be strength of solid matrix (i.e., ice formation; case hardening; surface cracks formation; permeability of water through crust; change in tertiary and quaternary structure of polymers; presence or absence of crystalline, amorphous, and viscoelastic nature of solids; matrix reinforcement; and residence time). However, some of these factors are related to the glass transition.

23.3.7 Microbial Stability

The microbial stability of food has long been estimated by its water activity. The rules are (i) the lower the water activity, the more microbiologically stable the food; and (ii) foods are most stable at its BET monolayer moisture content. The water activity at the monolayer water content is also called the *critical water activity*. One defect in this concept is that microbial stability is affected by the nature and type of solute at a given water activity. Another weakness is that water activity is defined at equilibrium, whereas foods at low and intermediate moisture are not in a state of equilibrium. In the dynamic state, water may be migrating from one component of food to another. This nonequilibrium state is difficult to predict by the equilibrium state defined by water activity.

Slade and Levine [126] and Franks [127] maintained that water activity could serve as a useful but not sole indicator of microbial safety. Slade and Levine's [126] hypothesis was that water dynamics or glass–rubber transition may be applied instead of water activity to predict the microbial stability of concentrated and intermediate-moisture foods. Sapru and Labuza [128] studied the inactivation of bacterial spores and their glass transition temperature. Spores at glass transition have high heat resistance, and above glass transition they are easy to inactivate. At a given temperature, the inactivation rate decreases with the increase of glass transition temperatures of spores. Chirife and Buera [129] maintained that glass–rubber transition would not be useful in predicting with confidence the microbial stability of foods. They analyzed data from the literature and concluded that water activity and glass transition are two different entities. The mobility factors (i.e., glass transition) in addition to water activity are not useful for a better definition of microbial stability of foods. Water activity is a solvent property and glass is a property related to the structure of food. Thus, both properties are needed for understanding food–water relationships at different conditions [129–131].

Macroscopic heterogeneities in a food material can induce the presence of areas with a higher mobility [57]. Chirife et al. [132] investigated microbial stability in glassy white bread and maltodextrins. They showed mold growth was possible below glass transition, if nonglassy microregions exist. Champion et al. [57] advocated for further studies to investigate effects of nonhomogeneous water distribution and/or phase separation on reaction rates. Hills et al. [133] first studied NMR relaxation and electrical conductivity to actually distinguish the effect of local rather than global water activity on microbial stress in porous media. They found that the microbial stress does not correlate with the global water activity measured for the whole assemble but rather with the local water activity of the water actually surrounding the cells.

23.3.8 Desiccation-Tolerant Organisms

Desiccation-tolerant organisms (anhydrobiotes), such as seed and pollen, are capable of surviving the removal of their cellular water. The lifespan of seeds can be remarkably long, ranging from decades to centuries [134, 135, 136] and even millennia [137]. In the late 1980s, Burke [138] forwarded the hypothesis that cytoplasm of seeds could enter into a glassy state. He suggested that in dry anhydrous organisms, glasses could be formed from cell solutes like sugars that were known to provide protection from denatured of large molecules and formation of molecular aggregates, and high viscosity may stop all chemical reactions that require molecular diffusion. Thus, the glass concept turned out to be an interesting hypothesis to account for the survival in the dry state. The physiological importance of the glassy state in desiccation tolerance and storage longevity was also assessed. More recently, in addition to the measurement of glass transition temperature, efforts focused on the assessment of additional physical properties, such as molecular density and local viscosity of the intracellular glassy matrix [12]. When the concept of glasses was introduced in seed science, sugars were thought to have an important part in composition and properties of glassy matrix. Many studies were conducted to explore the biological and physicochemical properties of the intracellular glassy matrix. Several techniques have been developed to provide further insights into the molecular properties of glasses, such as electron paramagnetic resonance (EPR) and Fourier transform infrared (FTIR) spectroscopy. Recently Buitinik and Leprince [12] reviewed these molecular properties of glasses. They considered that the protective effect is improved by hydrogen bonding, water replacement of the sugars with proteins, increasing T_g and T_c (collapse), and filling the small voids. A *perfect biological glass* would exhibit a high T_g and T_c, low molecular mobility, and high density, existing as a mixture of molecules that have high hydrogen-bonding capacity to undergo direct interactions with their neighboring molecules, as well as to prevent phase separation and crystallization [12].

23.3.9 Oxidation

Oxidation phenomena occur in low moisture food systems, such as fat or ascorbic acid oxidation. The oxidation of unsaturated lipids entrapped in sugar-based matrices is affected by physical changes such as collapse or crystallization occurring above glass transition [98, 139]. The encapsulated oil was released as a consequence of the crystallization of amorphous lactose. The released oil underwent rapid oxidation, while encapsulated oil remained unoxidized.

23.3.10 Nonenzymatic Browning

Phase transitions with physical aspects of the matrix are factors affecting the rates of nonenzymatic browning reactions [140–142]. Nonenzymatic browning below glass transition was very slow. The systems used were vegetables, dairy products, and model food systems with amino acids and sugars in a PVP matrix. Karmas et al. [141] showed that the rate of the reaction is low at temperatures below glass and increases with the increase of $T–T_g$. They also indicated that the reaction is also controlled by several other factors, such as structural changes and water content, independently of its plasticizing effect. In this case, both moisture and glass transition affected the reaction rate [140]. This is due to the changes in diffusion coefficient below glass transition when nonenzymatic reactions take place in the diffusion-limited region. Roos and Himberg [143] also showed that it is not stopped by the glass transition temperature of the maltodextrin, lysine, and xylose matrix, and is possible in the glassy state. The WLF equation was valid to predict the reaction rate constant as a function of moisture and temperature above glass [140].

There is not a general rule observed whether water activity or glassy state of the system as dictated by glass transition temperature impacts the rates of chemical reactions in reduced moisture solid food systems. Bell [144] studied the kinetics of nonenzymatic browning pigment formation in a model PVP (different molecular weight) matrix. The browning rates of matrices having different glass temperature, but constant water activity, were significantly different except when all were in the glassy state. As the system changed from a glassy state to a rubbery state, the rate of browning increased sevenfold. The rate of browning also increased as water activity increased from 0.33 to 0.54, but then appeared to plateau with further increases in water activity. In addition, the concentration of reactants in the aqueous microenvironment had a significant impact on the rate of brown pigment formation.

Bell et al. [145] studied the glycine loss and Maillard browning as a function of glass transition temperature. At water activity 0.54, pH 7, and storing at 25°C, the rate constants were very low when $T–T_g$ was close to zero and increased with the increase of $T–T_g$. O'Brien [146] studied the rate of nonenzymatic browning in freeze-dried model systems containing lysine with glucose or sucrose or trehalose at pH 2.5 and a water activity of 0.33. The temperatures were at 40°C, 60°C, and 90°C. All systems were in rubbery state at 90°C, whereas at 40°C and 60°C trehalose was in mixed amorphous glass–crystalline system, and glucose and sucrose were in rubbery state. The rate of nonenzymatic browning in the trehalose system was much lower than that in the sucrose or glucose depending on temperature. The rate constant was in the order glucose > sucrose > trehalose. The presence of crystalline material in the trehalose system at 40°C and 60°C may have influenced the overall rate of hydrolysis, stabilizing the system. At 90°C, all systems were in rubbery state and there were substantial differences between the stability of sucrose and trehalose. Thus, glass transition not only controls the rate of browning but also the rate-limiting sucrose hydrolysis step since glucose has a much higher rate than sucrose. The effect of glass transition was much less than previously reported. Karel et al. [88] developed correlation for the browning rate constant as a function of $1/T$, moisture content, and $T–T_g$.

Bell and Hageman [147] studied the kinetics of aspartame degradation in the PVP model system at constant temperature

(25°C) and pH (7.0) as a function of water activity and glass transition independently. Degradation reaction rates at constant water activity but different glass transition temperature were not significantly different, and rates at a similar distance from T_g but different water activities were significantly different. Thus, the rate of aspartame degradation was significantly influenced by the water activity, while the effect of the glass transition temperature on the reaction was negligible.

23.3.11 Enzymatic Reaction

Several enzymatic reactions can occur at low water contents [148, 149] or in the frozen state [25, 150–153] such as those catalyzed by alkaline phosphatase, lipoxygenase, lipase, or invertase. The effect of temperature on the reaction rate depends on the relative value of the diffusion of the reactants and the activity of the enzyme in such concentrated media. Champion et al. [57] pointed out that there is a risk in proposing a unified theoretical model to predict the reactions in such concentrated materials. Torreggiani et al. [154] found no clear relationship between the anthocyanin loss and $T–T_g'$ of strawberry juices. Other important factors such as the pH of the unfrozen phase could influence anthocyanin pigment stability. It could be hypothesized that sorbitol showing stability could alter the nucleophilic power of the water or could play a specific protective role, due to its chemical nature, in the enzymatic breakdown of the anthocyanin pigments.

23.3.12 Denaturation of Protein

The properties and functionality of the protein depend on whether it exists in the native or denatured state, and maintaining protein structure and functionality is important in food science. The kinetics of aspartame degradation were evaluated in the PVP model system [147, 155]. Reaction rates at constant water activity but different glass transition values were not significantly different. Moreover, rates at the same values of $T–T_g$ were significantly different with changing water activity. The temperature of denaturation decreased with increasing moisture content to some plateau, where further increases in moisture no longer influenced the denaturation temperature [156]. Bell and Hageman [147] studied the denaturation temperature of globular proteins (β-lactoglobulin, ovalbumin, and ribonuclease) in dry state as a function of glass transition temperatures of polyhydroxy compounds (water, glycerol, sorbitol, sucrose, and trehalose). The component was in the 25–33% w/w. They found thermal stability of protein correlated with glass transition temperatures of polyhydroxy component, and the lower the T_g of the component, the greater was the degree of protein destabilization. They hypothesized that in dry state, the additives were acting as plasticizers, enhancing the mobility and thus the unfolding of the globular proteins.

23.3.13 Hydrolysis

The effect of glass transition on different chemical reactions is not as clear as in the cases with physical changes. This is due to the multiple roles of water in foods, such as plasticizer, reactant or product of chemical reactions, and pH [157]. One of the chemical reactions that were proposed to occur only in the rubbery phase (i.e., above glass transition) is sucrose inversion in acid-containing amorphous powders [158]. Buera et al. [157] investigated the effect of glass transition on the rate of acid-catalyzed sucrose hydrolysis in an amorphous polymeric matrix of PVP. No direct relationship was found between sucrose hydrolysis in a PVP matrix and T_g or $T–T_g$. Glass transition is not a key factor determining the rate of sucrose hydrolysis. The major effect on the rate of hydrolysis was related to changes in pH, which is moisture dependent. Knowledge of the actual pH of a system, and the possible changes that may occur during concentration or drying are necessary for better understanding of chemical changes in low and intermediate moisture foods. Sucrose hydrolysis in an acid-containing (low pH = 3.1) amorphous starch powder (native or pre-gelatinized) occurred to a significant extent in the glassy state [159]. The mobility effects are not controlling the extent of reaction. Hydrolysis (31% to 85% remaining sucrose) was observed at different water contents and temperature below glass. Little reaction occurred at moisture contents below the so-called BET monolayer. Temperature was a critical factor controlling sucrose inversion.

23.3.14 Enzyme Inactivation and Other Chemical Reactions

The stability of enzymes in low-water systems was analyzed based on glass concept. The stability of lactase during heating at 70°C was studied in different amorphous glassy matrices: trehalose, maltodextrin, and PVP [160]. The protective effect of the maltodextrin and PVP matrices on the enzyme was attributed to their glass transition temperature, but the trehalose matrix is much more efficient for enzyme stability independent of its glass transition value. Schebor et al. [161] studied the stabilization of the enzyme invertase (β-fructofuranosidase) by its incorporation in aqueous model systems of trehalose, maltodextrin, and PVP, followed by freeze-drying and desiccation to zero moisture content. When the systems heated at 90°C for thermal inactivation of invertase, the enzyme was protected by maltodextrin and PVP, but not significantly protected by trehalose, although all systems were in the glassy state. Cardona et al. [162] observed significant inactivation of invertase when maltodextrin and PVP were kept well below their glass transition, but the enzyme was fairly stable in rubbery trehalose systems. At this moisture content, trehalose crystallization rapid thermal inactivation of invertase was observed. The invertase inactivation in heated systems of reduced moisture could not be predicted based on glass transition, and this was particularly true for trehalose (i.e., it was evident that the glassy state was not the main stabilizing factor).

The relevance of glass transition as a reference temperature for predicting the rate of chemical or enzymatic reactions was studied, but no clear relationship has been established [57]. Bell and White [163] studied thiamin loss as a function

of water activity and glass transition temperature. The maximum rate constant appeared to be around $T–T_g = –23°C$. Below glass transition (i.e., lower $T–T_g$), the rate constant decreased and correlated reasonably well with decreasing value of $T–T_g$. In the rubbery region (i.e., water activity more than 0.4) the rate constants no longer correlated with $T–T_g$ but rather with water activity. The reason could be attributed to the collapse of the matrix.

23.3.15 Vitamin C

Zhang et al. [164] studied the stability of vitamin C and other quality attributes (i.e., hardness, drip loss, and color) of mango (i.e., cube shape) within four different states of frozen storage (i.e., $T < T_g'$, $T_g' < T < T_g''$, $T_g'' < T < T_m'$, $T > T_m'$). They observed that vitamin C continued to decrease at the frozen glassy state even without temperature fluctuations. In addition, a higher rate of decrease was observed above frozen glassy state with temperature fluctuations. This indicated that the reaction rate could be slow in the glassy state but not zero. Therefore, when considering stability, it is important to identify the time frame. For example, if we are looking for 3 years of shelf life, then it needs to be considered what low rate would be good for the time frame of 3 years.

23.3.16 Sensory Properties

The effect of molecular weight on glass temperature of starch hydrolysis products (SHPs) shows a plateau region, which indicates the useful range of gelation, encapsulation, cryostabilization, thermochemical stabilization, and facilitating of drying process. The lower end corresponds to the area of sweetness, browning reactions, and cryoprotection. The intermediate region at the upper end of the steeply rising portion represents the area of antistaling ingredients. The map can be used to choose individual SHPs or mixtures of SHPs and other carbohydrates to achieve the desired complex functional behavior for specific product applications [16]. For example, the synthesis of SHPs capable of gelation from solution should be designed to yield materials of dextrose equivalent (DE) ≤6 and $T_g' \geq 8°C$. Similarly, potato starch maltodextrins of 5-6 DE (25% w/w) produced thermoreversible, fat-mimetic gels; and tapioca SHPs of DE ≤5 also form fat-mimetic gels from solution to develop fat-replaceable ingredients [165–167]. Levine and Slade [16] developed a linear relation between DE and glass transition temperature of a maximally freeze-concentrated solution of commercial SHPs. Thus, the correlation can be used to approximately calculate DE for dextrin and maltodextrin of unknown SHP, which can make unnecessary more tedious and time-consuming classical experimental methods for DE determination [168].

Glass transition alone could not be considered as generic rules for food stability criteria since numbers of instances, such as pore formation, diffusion, microbial stability, nonenzymatic browning, and other factors or mechanisms play important roles. However, it is definitely one of the factors affecting the stability, and a future challenge to combine the glass concept with other mechanisms or factors, such as water activity, pH, and preservatives [6].

23.4 CRITICAL TEMPERATURE CONCEPT

It is expected that there should be a break in the plot of k (i.e., reaction rate) versus T/T_g at T/T_g equal to 1 (i.e., change in slope between above and below glass transition), if the glass transition concept is valid. Similarly, a plot of k versus X_w/X_b should have a break at X_w/X_b equal to 1, if the water activity concept is valid. The molecular mobility measured by ST-EPR and ^{1}H-NMR showed two distinct changes: (i) a minor shift just close to T_g and (ii) an abrupt decrease due to a solid-like to liquid-like shift defined as T_c (i.e., critical temperature) [169]. In the case of sugars, T_c was observed at 17–35°C higher than T_g, and for biological materials it was more than 50°C. This variation was also explained by the density of hydrogen bond and molecular packing measured by FTIR. This higher T_c from T_g was also correlated with the observed collapse or softening of sugars at 10–17°C above glass transition [170–172] and crystallization onset above 22.1°C to 28.3°C higher than glass transition considering the level of moisture in date syrup [173].

In the case of browning of banana as a function of moisture content at different storage temperatures, the rate constant as a function of temperature (sample containing only unfreezable water, i.e., moisture: 0.04 g/g sample) is shown in Figure 23.3 [174]. Figure 23.3 shows a change of slope at a critical temperature, 45.0°C, which is higher than the onset glass transition (i.e., 11.7°C). This shows that the critical temperature is 33.3°C, which is higher than glass transition. The Arrhenius plot also shows a change in slope at 45.0°C, and this indicated that there was a change in reaction mechanisms above 45.0°C (Figure 23.4). Similarly, the deactivation of α-amylase in freeze-dried trehalose matrices (moisture: 0.50 g/g sample, temperature: 45–100°C) showed a change in the slope at 85°C, while the glass transition temperature was 45°C (i.e., 40°C higher than glass transition) [175]. However, the rate of browning in food systems showed a change in slope in the Arrhenius plot, and the critical temperature was observed either at glass transition or above/below the glass transition depending on the types of matrices [142]. In addition, in some instances the reaction rate

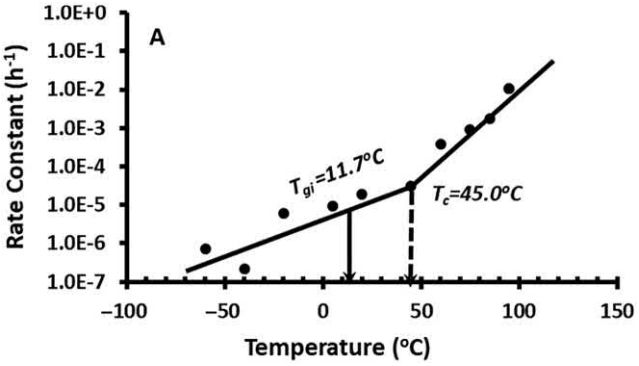

FIGURE 23.3 Plot of log k (reaction rate) as a function of temperature for the sample moisture 0.04 g/g sample. (From Rahman and Al-Saidi [174].)

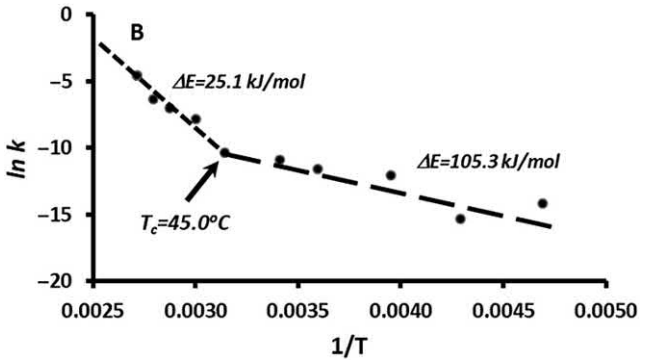

FIGURE 23.4 Arrhenius plot for the sample moisture 0.04 g/g sample. (From Rahman and Al-Saidi [174].)

increased in the glassy state instead of decreased as compared to the rubbery state. In the case of browning of banana, the activation energy values of freeze-dried banana below and above critical temperature were determined as 105.3 and 25.1 kJ/mole, respectively. Similarly, the activation energy of the decay of α-amylase in freeze-dried trehalose matrices (moisture: 0.50 g/g sample) showed 128 and 40 J/mole, respectively, below and above critical temperature [175].

In the case of browning of banana containing freezable water, a shift was observed when reaction rate was plotted as a function of temperature (i.e., two changes in the slope) (Figure 23.5). The first change in slope was observed close to the T_g''' (i.e., –40.9°C, critical temperature), thus supporting glass transition concept [174]. The enzyme decay in the frozen fish protein showed that the critical temperature existed at, below, or above T_g''', depending on the molecular weight of the used cryoprotectant (i.e., maltodextrin) [102].

Crystallization over a practical time scale occurred above glass transition, and some reports showed it was 30°C above glass temperature [176]. α-Amylase was more stable in rubbery matrices, of lactose or trehalose than in a glassy PVP matrix and the protective efficiency of saccharides, maltodextrins, and PVPs did not increase with their respective glass transition temperature [177, 178]. In addition, dielectric and other spectroscopy determined relaxations (i.e., β and γ) below glass transition [179]. However, it is not clear how these

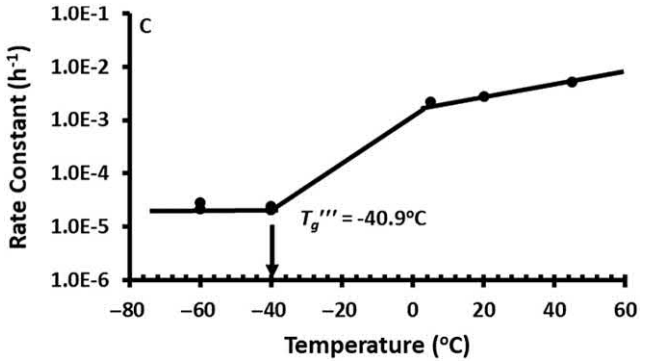

FIGURE 23.5 Plot of log *k* as a function of temperature for the sample moisture 0.869 g/g sample. (From Rahman and Al-Saidi [174].)

multiple relaxations linked to the stability of foods, as related to the physical and chemical changes.

Considering the fact that glass transition is not the critical limit, Rahman [3] tested the hypothesis that there is a critical temperature as a ratio of T_c/T_g (T_c is the critical temperature) that could vary with moisture content. Above the critical temperature, an increase in the water content or temperature significantly increased the reaction rate; while below the critical temperature, the rate was less affected by water content and temperature. Rahman [3] observed values of T_c/T_g that varied from 0.78 to 1.5 depending on the types of reaction and the matrices. In some instances, the values of T_c/T_g were close to 1.0 indicating that only in specific cases did glass transition explain the mechanism. Moreover, the deviations of T_c/T_g from 1 explain why in many instances in the literature both stability and unstability were observed above and below glass transition. Thus, the critical temperature in relation to glass transition depends on the types of chemical reactions as well as the physicochemical and structural characteristics of the matrices [174].

The molecular relaxation, as measured by different methods, determines relaxation at different scales (i.e., macro, micro, and nano ranges). The transition by thermal or mechanical relaxations measure mobility in a 20–300 nm range, while other relaxation techniques, such as NMR, measure the molecular relaxation in a 1–2 nm range [180]. Recently, tremendous emphasis has been given to understanding the multidimensional aspects of molecular mobility. Although, it is not yet very clear how this knowledge could be applied universally in determining the stability of foods in relation to microbial and physical-chemical changes.

23.5 CONCLUSION

It is clear from the literature that not all experimental results can be explained by the glass transition rules, thus further developments are necessary. The limitations of water activity and glass transition concepts do not invalidate the concepts completely but rather make them difficult to apply universally. The water activity concept is based on the binding nature of water molecules in the matrix. When water is bound (i.e., unavailable to take part in reactions) to the solid matrix or nonsolvent, then no deterioration reactions could be expected. The glass transition concept is based on the molecular mobility of the reacting components at microlevel in a matrix, thus diffusion of the reactants through the system is very slow and stability is achieved. Thus, a successful combination of water activity and glass transition concepts could open more precise and unified determination of stability criteria. There is a need to combine multiple concepts for better predictions of food stability.

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24 State Diagrams and Their Applications in Food Preservation

Mohammad Shafiur Rahman

CONTENTS

24.1 BACKGROUND AND PROGRESS

The glass transition concept was advanced further by the development of state diagrams for foods. A state diagram is a stability map of different states and phases of a food as a function of water or solids content and temperature. Most probably, Levine and Slade [1] presented the first state diagram in the food science literature by illustrating glass line, freezing curve, and the intersection of these lines as T_g'' by extrapolation of the extended freezing curve by maintaining similar curvature (Figure 24.1). The main advantage of drawing a map is to help in understanding the complex changes that occur when the solids content (or water content) and temperature of foods are changed. It also assists in identifying the stability of foods during storage as well as selecting suitable conditions in terms of temperature and moisture content for processing to achieve the desired product characteristics. This chapter provides the components of the state diagram by discussing macro- and micro-regions, and by explaining selected applications in determining food's stability during processing and storage.

24.1.1 Macro-Region State Diagrams

The initial state diagram based on the freezing curve and glass transition provided four macro-regions: region I (i.e. below the glass transition), region II (above the glass transition and below the maximal freeze concentration, i.e. completely frozen), region III (above the maximal freeze concentration condition and below the freezing curve, i.e. partially frozen), and region IV (i.e. above the glass transition and above the freezing curve) [1] (Figure 24.1). It is important to understand the state diagram first before applying it in food processing and food storage.

24.1.2 State Diagrams Combining Water Activity and Glass Transition

Recently many papers have presented data on the water activity as well as glass transition as a function of water content. However, it was not clearly identified where the link was between them in order to determine stability. Karel et al. [2] attempted to relate water activity and glass transition by plotting equilibrium water content and glass transition as a function of water activity. By drawing a vertical line on the graph, stability criteria could be determined from the isotherm curve and glass transition line. At any temperature (say 25°C), the stability moisture content from the glass transition line was much higher than the stability moisture from the isotherm. However, this approach is unable to relate this to experimental food stability. At present, it is a real challenge to link them.

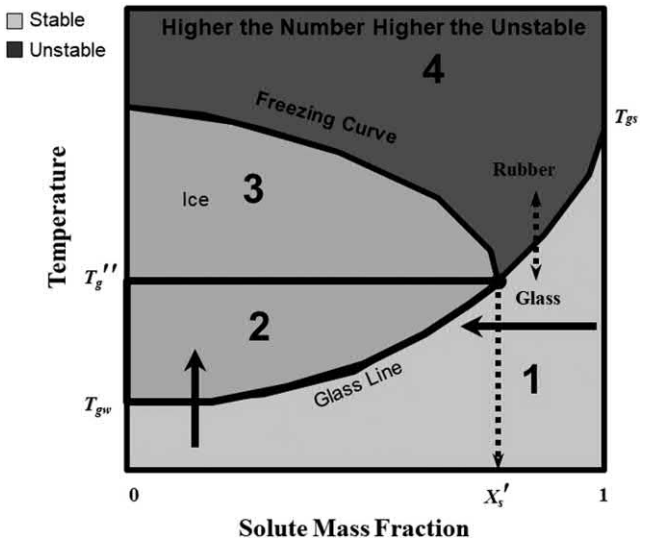

FIGURE 24.1 Macro-region state diagram; 1, 2, 3, 4 indicate the degree of stability, 1: most stable and 4: least stable. (From Levine and Slade [1].)

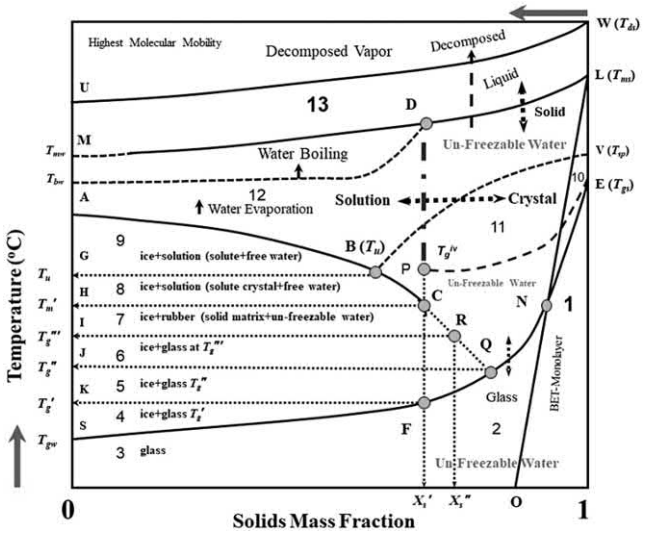

FIGURE 24.2 State diagram showing different regions and state of foods (updated from [3–6]) T_{ds}: solids-decomposition temperature, T_{ms}: solids melting temperature, T_{sp}: soluble solids melting, T_{gs}: solids–glass transition temperature, T_g^{iv}: end of solids-plasticization temperature, T_u: eutectic temperature, T_{gw}: glass transition of water, T_u: solute crystallization temperature during freeze-concentration, T_m': maximal-freeze-concentration condition, i.e. end point of freezing, T_g''': glass transition of the solids matrix in the frozen sample as determined by differential scanning calorimetry (DSC), T_g'': intersection of the freezing curve to the glass line by maintaining the similar curvature of the freezing curve, and T_g': glass transition at maximal-freeze-concentration, i.e. at the end point of freezing, T_{bw}: boiling temperature of water, T_{mw}: extension of solids-melting line to y-axis.

Most approaches to combining both concepts are reviewed by Rahman [3, 4].

Rahman [5] combined the water activity and glass transition concepts in the state diagram by plotting the BET-monolayer as a function of temperature. This makes four macro-regions: above freezable (i.e. unfreezable region) moisture content; below the BET-monolayer, one above and one below; and above the BET-monolayer, one above and another below (Figure 24.2). In the literature, it was emphasized that a combination of the water activity and glass transition concepts could be a powerful tool in predicting food stability. A successful combination of water activity and glass transition could lead to more in-depth knowledge on stability criteria [5]. Recently, attempts have been made to add other structural changes, such as glass line, freezing curve, and solubility line, to the state diagram.

24.1.3 Micro-Regions in the State Diagram

Using a state diagram, Rahman [6] hypothesized 13 micro-regions with the highest to the lowest stability based on their location relative to the glass and BET-monolayer lines. Figure 24.2 presents the most advanced state diagram. For example, region 1 (a relatively non-reacting zone, below the BET-monolayer line and glass line) is the most stable, and region 13 (a highly reacting zone, far from the BET-monolayer line and glass line) is the least stable. The stability decreases as the zone number increases. Table 24.1 shows the states or phases in different micro-regions. Applications of this hypothesis in food processing are presented by Rahman [3, 4, 6]. Schebor et al. [7] studied sucrose hydrolysis in the micro-regions 1 and 2, and their results evidenced the validity of the hypothesis (i.e. the reaction rate in micro-region 2 is higher than in micro-region 1). The advantages of the micro-region concept are as follows: (i) stability rules could be developed for each micro-region (i.e. narrow moisture and temperature range) as compared to the macro-region (i.e. broad moisture and temperature region), and (ii) the states or phases of the material could be identified in each micro-region, and (iii) a reference point could be identified where the BET-monolayer line and glass line intersect, and any location in the state diagram could be assessed in relation to the reference point [3, 4, 8].

TABLE 24.1
State Diagram Developed for Different Foods and Food Components

Material	Components	References
Apple, grape, onion, strawberry, sucrose, fructose, bacterial suspension, tomato, garlic	Freezing curve, glass line, X_s'', and T_g''	[19, 35, 59–68]
Honey	Freezing curve, glass line, X_s'', T_g'', and T_g'''	[69]
Sucrose	X_s'', T_g''	[70]
Sucrose, lactose, maltose, glucose, maltodextrins, starch, arabinoxylan	Freezing curve, glass line, X_s', T_m', and T_g'	[30, 71–74]

In the case of banana, Rahman and Al-Saidi [9] identified the crossing point of the glass line and BET-monolayer line at X_s equal to 0.92 g/g solids, and T_r (i.e. crossing point of glass and BET-monolayer line) equal to 1.4°C. However, the methods of combining other hurdles such as pH and preservatives could be linked with these concepts. We are far away from developing a unified theoretical basis combining multi-hurdles or multi-mechanisms of food stability.

24.2 MICRO-REGION STATE DIAGRAMS AND THEIR COMPONENTS

This section presents different characteristics of micro-region state diagrams and the prediction models of the phase or state boundary. Examples of recent state diagrams for broccoli (Figure 24.3, [10]), banana (Figure 24.4, [9]), and crystallized date fruit syrup (Figure 24.5, [11]) are presented in the literature. Table 24.1 shows other sources of state diagrams for foods.

24.2.1 Freezing-Related Process

In Figure 24.2, the freezing line (ABC) and solubility line (BV) are shown in relation to the glass transition line (EFS).

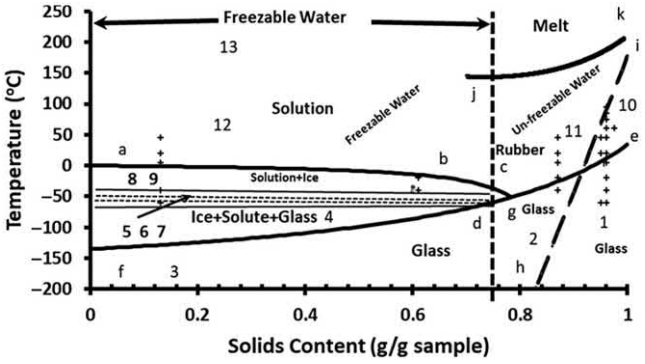

FIGURE 24.3 State diagram of freeze-dried broccoli; numbers show the locations of the micro-regions. (From Suresh et al. [10].)

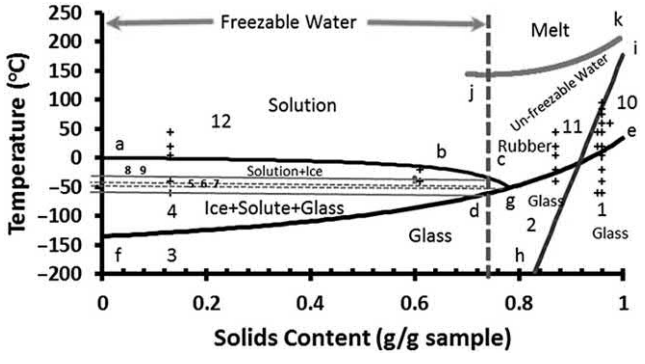

FIGURE 24.4 State diagram of ripe banana; abcg: freezing curve, fdge: glass line, c: ultimate maximal freeze concentration condition, d: vertical line passing through maximal-freeze concentration condition and crossing glass line, g: intersection of the freezing curve keeping the same curvature as the Chen [5] model and glass line, hi: BET-monolayer line, jk: solids-melting line [9].

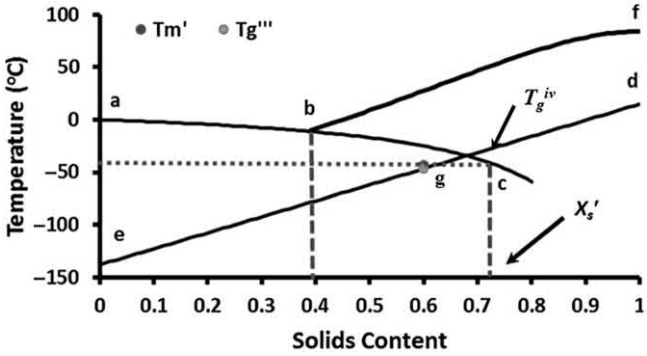

FIGURE 24.5 State diagram of crystallized date syrup (abc: freezing curve, g: T_m', de: glass line, c: maximal freeze concentration conditions, bf: sugar crystals melting line, b: eutectic point). (From Al-Farsi et al. [11].)

The freezing line decreased with the increase of solids due to the freezing point depression. The cooling curve is one of the most simple and popular methods to measure the freezing point of foods. The complete discussion is not presented here since details of the cooling curve method are given by Rahman [12] and Rahman et al. [13]. The cooling curve method was used to measure the freezing point of milk [14, 15], coffee extract [16], dates [17], tuna flesh [18], and garlic [19].

A typical DSC heating thermogram of fruits and vegetables is shown in Figure 24.6 for a sample containing freezable water [20]. The sample containing freezable water shows two shifts, one at low temperature (i.e. the first glass transition, marked as G_1) and another one (i.e. the second glass transition, marked as G_2) just before the ice melting endothermic peak (i.e. marked as M). The values of maximal-freeze-concentration conditions (i.e. T_m' and T_g''') are determined from the second transition as shown in Figure 24.2. The freezing point and ice melting enthalpy can be determined from the endothermic peak. The initial or equilibrium freezing point was considered as the maximum slope in the ice melting endotherm (marked as m) as suggested by Rahman [20]. The ice melting (i.e. freezing point) is commonly characterized from the endothermic peak during melting [20, 21]. This method provides very accurate determination for a sharp peak. In the case of a wider peak, the freezing point is difficult to determine. The wider peak appears due to the wide variation in the state of water in foods. In this case, the maximum slope of the endotherm (point m in Figure 24.6) or the extra-plotted peak onset temperature of the ice melting can be considered the freezing point [21, 22]. When the sample contains mainly free water, it shows a sharp endothermic peak on melting at a melting point similar to pure water. Multi-peak natures of the DSC curves are found for the metastable states of water in gum from *Acacia senegal* [23] and gellan [24]. The sample containing unfreezable water shows no first-order transition (Figure 24.7) for ice melting, instead only showing a solids-melting endotherm.

The extended theoretical-based Clausius–Clapeyron equation is commonly used to model the freezing point of foods as a function of solids content [25]. The improved model is

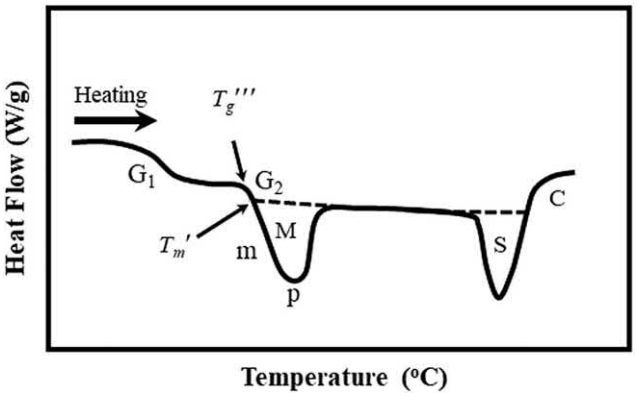

FIGURE 24.6 A typical DSC thermogram for the sample containing freezable water. M: ice melting peak, S: solids-melting peak, G_1: lower glass transition, and G_2: glass transition just before ice melting, T_m': end of ice formation, and T_g''': onset and end of G_2. (From Rahman [58].)

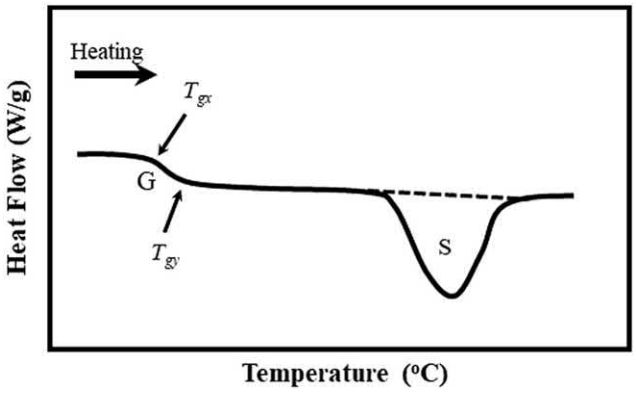

FIGURE 24.7 A typical DSC thermogram for the sample containing unfreezable water. G: glass transition, S: solids-melting. (From Rahamn [58].)

adapted for non-ideal solutions by introducing parameters for the fraction of total water unavailable for ice formation. The unfreezable water content B (g water/g dry-solids) can be defined as the ratio of unfreezable water to the total dry-solids. The unfreezable water is defined as the water which never forms ice even at very low temperatures (e.g. –40°C). The Chen [25] model can be written as:

$$\delta = -\frac{\beta}{\lambda_w}\ln\left[\frac{1 - X_s^o - BX_s^o}{1 - X_s^o - BX_s^o + EX_s^o}\right] \qquad (24.1)$$

where δ is the freezing point depression, $\delta = (T_w - T_F)$, T_F is the freezing point of food (°C), T_w is the freezing point of water (°C), β is the molar freezing point constant of water (1860 kg K/kg mole), λ_w is the molecular weight of water, X_s^o is the initial solids mass fraction before freezing (g/g sample), B is the unfreezable water (g/g dry solids), and E is the molecular weight ratio of water and solids (λ_w/λ_s). The values of model parameters B and E for different foods are shown in Table 24.2.

The point F (X_s' and T_g') lower than T_m' (point C) is a characteristic transition (ultimate maximal freeze concentration condition) in the state diagram, and it is defined as the intersection of the vertical line from T_m' to the glass line EFS [5]. The water content at point F or C is considered the unfreezable water ($1 - X_s'$) (i.e. water mass fraction which remained unfrozen even at very low temperatures, e.g. –40°C). It includes both uncrystallized free water and bound water attached to the solids matrix. The point Q is defined as T_g'', and X_s'' as the intersection of the freezing curve and the glass line by maintaining the similar curvature of freezing curve. Matveev [26] proposed a method for estimating the T_g'' and X_s'' intersection point in the state diagram of a frozen solution using the glass transition temperature of the solute.

Point R is defined as T_g''' as the glass transition of the solids matrix in the frozen sample, which is determined by DSC considering ultimate annealed conditions (Figure 24.6). This is due to the formation of the same solid matrix associated with unfreezable water and the transformation of all free water into ice although the sample contains a different level of total water before the start of DSC scanning [19]. This specific procedure needs to be followed to determine the ultimate maximal-freeze-concentration conditions (ultimate T_m', T_g''') when DSC protocols are used. First, samples (i.e. containing freezable water, 0.05–0.6 g/g sample) need to be cooled from 25 to –90°C at 5°C/min and then heated to the desired end temperature at 5–10°C/min (i.e. depending on what the temperature thermal characteristics are intended to determine, 60–300°C) (Run 1). Rahman et al. [27] experimentally showed that 10°C/min was the best for measuring the freezing point, maximal-freeze-concentration condition, and enthalpy. The freezing point and apparent maximal freeze concentration (apparent T_m', T_g''') are determined (Figure 24.6). In this practical cooling and heating step, all freezable water would be unable to form ice during cooling and all ice would not be melted during heating, since crystallization and melting are kinetic processes. In the second step, the protocol is as follows: samples (i.e. containing freezable water, 0.05–0.6 g/g sample) need to be cooled from 25°C to apparent $T_m' - 1$ (i.e. if apparent T_m' is –40°C, then annealed at –41°C) for 30 min, and samples are then again cooled to –90°C at 5°C/min and then heated to the desired end temperature at 5–10°C/min (i.e. depending on what the temperature thermal characteristics are planned to determine, 60–300°C) [20, 28]. This protocol provides the annealed maximal-freeze-concentration (annealed T_m', T_g'''). The annealed T_m' and T_g''' decreased with the increase of solids [19]. In most of the cases, the annealed T_m' and T_g''' remained constant after X_s above 0.6 g/g sample (i.e. moisture 0.4 g/g sample) [19]. This end point could be experimentally determined or T_m' and T_g''' for the sample at solids 0.6 g/g sample (or experimentally determined optimum solids) could be considered as ultimate freeze-concentration condition and used in the state diagram as shown in Figure 24.2. Al-Farsi et al. [11] observed that annealed T_m' and T_g''' increased after solids content 0.6 g/g sample, instead of decreasing as expected due to the freezing point depression with increasing solutes content. This indicated the optimum traceable condition to determine the uninterrupted ice melting, and above solids 0.6 g/g sample, the matrix would be very

TABLE 24.2
Characteristic Points of the State Diagram and Gordon–Taylor and Gordon–Taylor–Rahman Models Parameters for Different Foods

Material	X_s'	B	E	X_s''	T_m' (°C)	T_g''' (°C)	T_g' or T_g^{iv} (°C)	T_g'' (°C)	T_{gs} (°C)	T_{gw} or T_c	k_g or k_c	Reference
Artificial rice[2]	0.760	0.023	0.050	-	–8.3	–8.4	29.8	-	75.5	29.80	6.40	[40]
Banana[1]	0.740	0.073	0.162	0.760	–34.5	–40.8	–65.0	–52.0	34.2	–134.70	3.68	[9]
Broccoli[1]	0.700	0.199	0.095	0.740	–30.0	–32.0	–73.5	–68.5	42.8	–134.70	4.30	[10]
Date fruit[d]	0.762	0.053	0.129	-	–43.6	–46.4	–46.5	-	63.8	–134.70	4.00	[20]
Date syrup[1]	0.730	0.020	0.177	-	–42.8	–46.5	–20.0	–46.5	14.9	–134.70	1.01	[11]
Gelatin[1,c]	0.610	0.450	0.120	-	–47.3	–52.6	–56.0	-	154.0	–135.00	0.22	[75]
Gelatin[2,b]	0.800	0.510	0.060	-	–11.9	–14.9	34.0	-	153.7	6.80	17.30	[3]
Gelatin[2,b]	0.620	0.510	0.060	-	–46.9	–52.9	–58.0	-	194.0	–135.00	0.17	[75]
Mango[1]	0.160	–0.061	0.082	0.830	–33.0	–43.2	–54.6	–43.2	12.2	–134.70	4.50	[45]
Skin extract[1,a]	0.520	0.732	0.075	0.560	–32.2	–33.4	–76.0	–72.0	1.5	–134.70	1.20	[76]

[1] Gordon–Taylor Model
[2] Gordon–Taylor–Rahman Model
[a] Pomegranate
[b] Bovine
[c] Salmon
[d] Khalas

viscous, and it would take much longer to crystallize all water. Table 24.2 shows the compiled ultimate T_m' and T_g''' values for different foods.

Below the region ACH, the phases present are ice and rubber (i.e. matrix of solids and unfreezable water). Below point B, the first crystallization of solute occurs; thus the HCBG region transforms into three states: ice, solution, and solute crystals. There is no free water (i.e. water able to form ice) existing on the right side of point C (T_m', end point of freezing with ultimate maximal freeze-concentration-condition), and after this point the very concentrated solution is transformed to glass–rubber transition only without freezing. The ultimate maximal-freeze-concentration condition could be achieved using optimum conditions by slow cooling and annealing of the samples as mentioned earlier. The region IRCH contains ice, rubber, and solute crystal. The point R is the T_g''' (as shown in Figure 24.2); below this point a portion of the rubber state is transformed to the glass state, and thus the region JQRI contains glass, ice, and solute crystal. The point F is the vertical line passing through point C and crossing the glass line (i.e. T_g'). The rate of cooling can shift the points B, C, R, Q, and F. Therefore, specific procedures must be used for ultimate maximal freeze concentration and freezing point as discussed earlier. More detailed effects of cooling on the shift are discussed by Rahman [20].

24.2.2 Glass Transition Line

The regions BQEV and BVLD are important in food processing and preservation, since many characteristics such as crystallization, stickiness, and collapse are observed in these regions [29, 30]. In the case of cereal proteins, Kokini et al. [31] determined the entangled polymer flow region when both G' (i.e. storage modulus) and G'' (i.e. loss modulus) decreased with the increase of temperature and showed a minimum peak. A reaction zone was defined when both G' and G'' increased from the minimum peak and started to separate from each other and then decreased again starting the softening region. All these transitions observed in the region BQEV and the lines of entangled, reaction, and softening regions could be drawn as a function of solids content in the state diagram. This could identify additional states or phases in the state diagram beyond the glass line, such as solids-melting and soluble-solids-melting lines.

A typical thermogram for a sample containing unfreezable water is shown in Figure 24.7. It shows a shift for the glass transition (marked as G) followed by an endothermic peak for solids-melting (marked as S). Line EQFS is the glass line when onset glass transition is plotted as a function of solids contents (Figure 24.2). Glass transition decreased with the increase of solids (i.e. plasticization with the increase of water). Glass line modeling is important in identifying the glass–rubber transition boundary. Considering the free volume concept and that the effect of free volume is additive, the glass transition temperature of a polymer–water system can be written as [32]:

$$T_{gm} = \frac{\varepsilon_s T_{gs} + \phi \varepsilon_w T_{gw}}{\varepsilon_s + \phi \varepsilon_w} \tag{24.2}$$

where, T_{gm}, T_{gs}, and T_{gw} are the glass transition temperatures of the mixture, dry solids, and water (°C); ϕ is the volume expansion coefficient difference of polymer and diluent; and ε_w and ε_s are the volume fractions of water and solids or polymer, respectively. The influence of water content on the glass transition temperature is commonly modeled by the Gordon–Taylor equation [33]:

$$T_{gm} = \frac{X_s T_{gs} + k_g X_w T_{gw}}{X_s + k_g X_w} \tag{24.3}$$

where k_g is the Gordon–Taylor parameter. The values of k_g are compiled in Table 24.2 for different foods. This equation is valid if the glass transition follows the line EQFS in the state diagram (i.e. maximum plasticization). If experimental data do not follow the line EQFS in Figure 24.2, the interaction deviates from the Gordon–Taylor model at low moisture (for example, line EP). In this case, Rahman [6] proposed the modified Gordon–Taylor–Rahman model as:

$$T_{gm} = \frac{X_s T_{gs} + k_c X_w T_c}{X_s + k_c X_w} \tag{24.4}$$

In the above equation, T_c is considered as critical temperature (°C) instead of T_{gw}, and it is related to $T^{iv}{}_g$ (i.e. point P, vertical line passing through C and F at X_s' and crossing glass line EP) as defined in the state diagram as shown in Figure 24.2 [27]:

$$T_c = T_g^{iv}\left(1 - X_s\right) \tag{24.5}$$

This correction is needed since the origin of Gordon–Taylor equation is now shifted from 0 to X_s'. The point $T^{iv}{}_g$ is on the same vertical line as T_g' and T_m'. The parameter, $T^{iv}{}_g$ is compiled in Table 24.2.

24.2.3 Solids Melting Line

The line LDM is the melting line, which is important when products are brought to high temperatures during processing, such as frying, baking, roasting, or extrusion cooking. In the case of multi-component mixtures, such as food, the melting peak temperature shifted with the heating rate due to the reactions between components when the heating rate is varied (i.e. total annealed time is varied). In this case, it is considered as apparent melting. Below LDM, solid and liquid phases are present, and above only the liquid phase is present. In foods, some (for example starch and gelatin) show melting phase changes from solid to liquid (i.e. above line LD); however many foods (for example bran and cellulose) do not transform from solid phase to liquid phase; instead these decompose from solids to vapor with solids residue (i.e. directly below LD to above WU). The line WU is defined as the decomposition line as material decomposes to vapor with or without solids residue. The line AD is the water boiling line below

the solids melting (line MD). This line does not intersect the y-axis at the right since boiling is difficult to observe in very concentrated solutions, where the water is strongly bound with solids. The solids-melting line LDM (i.e. loss of crystals or molecular order) is commonly modeled by the theoretical Flory–Huggins equation as [34]:

$$\frac{1}{T_{mp}} - \frac{1}{T_{ms}} = \left(\frac{R}{\Delta H_u}\right)\left(\frac{V_u}{V_w}\right)\left(\varepsilon_w + \chi\varepsilon_w^2\right) \tag{24.6}$$

where T_p and T_s are the peaks of melting temperature for the polymer (i.e. crystallized date syrup) with diluent (i.e. water), and for pure polymer (i.e. only dry solids) (K) respectively, R is the gas constant (8.314 J/g mole K), ΔH_u is the heat of fusion for repeated polymer units in the diluent (J/g), V_w is the molar volume of the diluent (m^3/g mole), V_u is the molar volume of polymer unit (m^3/g mole), ε_w is the volume fraction of the diluent, and χ is the Flory–Huggins polymer–diluent interaction parameter. Equation 24.6 was first developed independently by Flory [35, 36] and Huggins [37] from the thermodynamics of (binary) regular polymer solutions. The model was described in great detail by Flory in his famous book *Principles of Polymer Chemistry* [34]. The volume fraction of water in Equation 24.6 can be calculated from the following equation considering the volume of water and volume of solids estimated from the mass fractions and density values of water and solids [38]:

$$\varepsilon_w = \frac{\left(X_w/\rho_w\right)}{\left(X_w/\rho_w\right) + \left(X_s/\rho_s\right)} \tag{24.7}$$

where X_w is the mass fraction of water (g/g sample), ρ_w is the density of water (kg/m^3), X_s is the mass fraction of solutes content (g/g sample), and ρ_s is the density of dry solids (kg/m^3). It is assumed in Equation 24.7 that excess volume due to mixing of water and dry solids is negligible. The values of T_{ms}, $RV_u/\Delta H_u V_w$, and χ for broccoli were estimated as 178.4°C, 5.07×10^{-4}, and 0.69, respectively [10]. The values of χ were reported as 2.2 for gelatin (Rahman et al. 2010), 0.48–0.5 for starch [39], 0.0088 for artificial rice [40], and 0.0068 for date-pits [41]. The value of χ indicates the water–solids interaction during the melting process. The low value indicates the low power of the solvent (i.e. water) to interact with the solids while molecular degradation progresses within the melting process. This indicates that stiff molecules (e.g. artificial rice and date pits) show low interaction with the water molecule during the melting process. In the case of broccoli, the value of 0.69 as compared to high (i.e. 2.2) and low (i.e. 0.0068) values indicated that water moderately interacted with the solids during its melting process [10].

24.2.4 Crystal Melting Line for Soluble Solids

The line BV is the crystal-melting line and is mainly identified in the case of solution with dissolved solute(s). The right side indicates that solute crystal(s) is formed from solution and the left side indicates that crystal(s) is dissolved within the solution. Al-Farsi et al. [11] developed the crystal-melting line for date syrup and modeled the peak temperature by the Flory–Huggins equation as shown in Equation 24.6. The eutectic conditions (i.e. eutectic temperature and eutectic solids content, T_u and X_{su}) were determined from the intersection point of the crystals-melting curve as modeled by the Flory–Huggins equation and the freezing curve as modeled by the Chen [25] model. The T_{sp} (i.e. soluble solids melting) of pure crystal (i.e. only dry solids) was estimated as 84.0°C (point f in Figure 24.5), $[(R/\Delta H_u)/(V_u/V_w)]$ was estimated as 3.583×10^{-8}, and the Flory–Huggins polymer–diluent interaction parameter, χ, was estimated as 5.438×10^4, respectively. The eutectic point of date syrup was estimated from Figure 24.5 as T_u equal to –10.2°C (i.e. b in Figure 24.5) and eutectic solutes content 0.39 g/g sample. Similarly, the eutectic points of orange and bitter gourd juices were observed as –10.0°C [42] and –37.5°C, respectively [43]. However, the eutectic temperatures of α-D-glucose and β-D-glucose were shown to be –4.9°C (eutectic solutes 0.31 g/g sample) and –21.0°C (i.e. eutectic solutes 0.62 g/g) [44].

24.2.5 BET-Monolayer Line

Rahman [5] plotted the BET-monolayer value as the LNO line in the state diagram as shown in Figure 24.2. It intersects at point N with the glass line EQS, which shows that at least in one location (point N) glass and water activity concepts provide the same stability criterion. This approach forms more micro-regions, which could give different stability in the state diagram. Suresh et al. [10] developed a linear regression equation as:

$$T_b = 1003.2\,X_{bs} - 903.6 \tag{24.8}$$

where T_b is the temperature (°C) at BET-monolayer moisture, and X_{bs} is the solids content (g/100 g sample) at the BET-monolayer, respectively. This equation shows that the point L (Figure 24.2) is 99.6°C when solids content is 1. Equation 24.8 is used to develop the BET-monolayer line in the state diagram [5, 45] (i.e. line LNO). Similarly, Rahman and Al-Saidi, [9] developed an equation for ripe banana:

$$T_b = 2198.2X_{bs} - 2020.9 \tag{24.9}$$

The above equation shows that the BET-monolayer approached zero at 178.2°C (i.e. X_{bs} equal to 1). Rahman and Al-Saidi [9] defined the isokinetic temperature (T_{is}) (i.e. the temperature at which all reactions of the series proceeded at the same rate) from the linear plot of the slope of enthalpies (ΔH) and entropies (ΔS) according to Exner [46]. At specific moistures (i.e. M_w: 0.1, 0.2, 0.3, and 0.4 g/g dry-solids), the enthalpies ($\Delta H/R$) and entropies ($\Delta S/R$) were estimated from the slopes and intercepts of the linear plots (i.e. ln a_w *versus* 1/T), according to the following Equation 24.10 [47]:

$$\ln a_w = \frac{\Delta H}{RT} - \frac{\Delta S}{R} \tag{24.10}$$

Where a_w is the water activity, and ΔH and ΔS are enthalpy (kJ/kg) and entropy (kJ/kg), respectively. The isokinetic temperature was then estimated from the slope of the plot of enthalpies ($\Delta H/R$) and entropies ($\Delta S/R$) [46, 47]. The isokinetic temperature was estimated as 192.4°C for banana [9], which is close to the T_{bs} (i.e. 178.2°C). In the case of mango, the isokinetic temperature was estimated 228.0 and 190.0°C from desorption and adsorption isotherms [47]. Table 24.3 summarizes the states or phases in different micro-regions.

24.3 APPLICATIONS OF MACRO-REGION AND MICRO-REGION IN FOOD PROCESSING

24.3.1 Stability and Chemical Reaction

It is important to determine the applications of the macro- and micro-regions for stability or reactivity within different phase or state boundaries [5, 8]. Rahman and Al-Saidi [9] tested the validity of the macro-micro-region concept in the state diagram. They considered the browning of raw and freeze-dried banana slices as a function of moisture content and storage temperature (i.e. at different micro-regions). The reaction was modeled by first-order chemical reaction, and the variation of the reaction rate constant was analyzed based on the glass transition, water activity, and macro/micro-region concepts. The rate constants are plotted as a function of micro-region (Figure 24.8), and it shows an increasing trend, which supports the proposed concept (i.e. reaction rate increases as the micro-region number increases). However, there are variations of rate constant at each micro-region, which is expected as the moisture and temperature also varied in each micro-region.

TABLE 24.3
Phase/State and Other Characteristics of Foods in the Different Micro-Regions

Micro-Region	Phase/State and Other Characteristics
1	Solids, glass, strongly bound water
2	Solids, glass, unfreezable water
3	Solids, fast-cooled glass, unfreezable water, ice
4	Solids, slow-cooled glass-T_g', unfreezable water, ice
5	Solids, slow-cooled glass-T_g'', unfreezable water, ice
6	Solids, slow-cooled glass-T_g''', unfreezable water, ice
7	Solids, slow-cooled ice-T_m', unfreezable water
8	Solids, slow-cooled ice, unfreezable water, solute crystal-T_u
9	Solids, slow-cooled ice, unfreezable water, solution (dissolved solids and freezable water)
10	Solids, rubbery, bound water, unfreezable water
11	Solids, rubbery-T_g', unfreezable water
11	Solids, rubbery-T_g^{iv}, unfreezable water
12	Solids, solution (dissolved solids, freezable water, unfreezable water)
13	Solids, solution (dissolved solids, freezable water, unfreezable water), vapor

Source: Rahman [58].

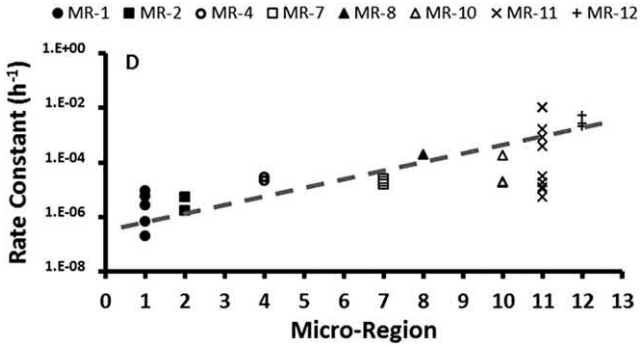

FIGURE 24.8 Plot of log k as a function of micro-region. (From Rahman and Al-Saidi [9].)

Rahman and Al-Saidi [9] pointed out that this plot could not solve the problem completely, but it would help in developing generic rules in each micro-region (i.e. smaller range of moisture and temperature within the same phase or state) rather than macro-region (i.e. wider range of moisture and temperature having different phases and states).

24.3.2 Drying

The glass–rubber transition is central for the successful use of the freezing and drying process as these are visualized in the state diagram [3, 4, 48]. In addition, the final state of the products could be located to determine their stability. Figure 24.9 shows the dynamics of the drying process in the state diagram. Point a in Figure 24.9 represents fresh foods with a high moisture content at room temperature before drying. The air-drying path is shown by the paths abc, abcd, or abcdg. The sample temperature is increased to wet bulb temperature during the progress of drying and solids content is increased due to loss of moisture. At the end of drying, sample is reached at a point after cooling, i.e. c or d as a rubbery state or g as a glassy state. After cooling the dried sample reaches the glassy state at point f if it follows the path of df, and it becomes stable. Alternatively, it could reach point e as unstable rubbery

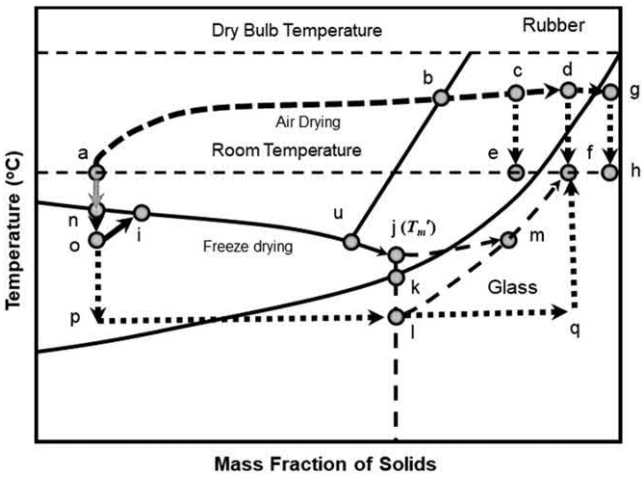

FIGURE 24.9 Paths of air- and freeze-drying processes in the state diagram. (From Rahman [3, 4].)

state, and the product is unstable. If drying ends at point g then it reaches the glassy state even during the drying process and cooling moves the sample even much lower than the glassy state (i.e. point h).

Collapse or shrinkage occurs in the sample since the air-drying process occurs through the rubbery state. The extent of collapse depends on the air-drying temperature and exposure time in the rubbery state. For example, in the cabinet dryer, the process takes in the order of days, while spray-drying may take in the order of minutes. Thus, in spray drying the quality deterioration is usually low due to the short exposure time. In addition, the time from b to c, or b or d, or b to g is also important since a number of deteriorative changes, such as crystallization, collapse, stickiness, and deteriorative reactions, could occur in the rubbery state. When the product reaches point b, soluble solute(s) is transformed to crystals (for example, sucrose). In addition to time and temperature, pressure could play a significant role, for example, at the low pressure used in vacuum and freeze-drying processes, gases and water vapor could form bubbles inside the product.

In the case of freeze-drying, product is first cooled from point a to n and ice crystal is formed (i.e. ice nucleation). The freezing process continues and follows the path iuj while the amount of ice is increasing due to the formation of ice, causing freeze-concentration in the liquid aqueous phase (if the freezing process is relatively slow). If the freezing process is very fast, then the sample is moved from a to p. After the freezing process, the pressure on the sample is reduced below 612 Pa so that the sublimation process can start rather than melting of ice. Sublimation starts at point p if it is the freeze-drying temperature, and the sample moves from p to l (i.e. end of sublimation), and at the end of freeze-drying, it reaches point q. In this case freeze-drying occurs below the maximal freeze concentration and maximum ice formation (i.e. T_m' or T_g'''), and minimal collapse could occur when ice sublimates. This is due to the glassy matrix around pores (i.e. formed due to ice sublimation) which would be able to support its own weight against flow under gravitational stress and so maintains its porosity (i.e. less collapse) [48].

In the case of slow cooling the sample reached point l from j (i.e. freeze-drying temperature) and sublimation continued. It is important to mention that point p to l is the sublimation process and point l to q is the vacuum drying (i.e. desorption) of unfreezable water at low pressure, since after point l there is no ice to sublimate. The characteristics of the final freeze-dried materials depend on the freeze-drying temperature (i.e. below or close to T_m' or T_g''') and the time to reach from point a to f. If freeze-drying occurs at T_m' or a higher temperature, then the sample will follow j to m. If the freeze-drying temperature continuously increases above T_m' or T_g''', then the sample will follow the path jmf or lmf. It is important to mention that the collapse temperature usually occurs above T_g'''. For example, microscopic observation showed that the onset collapse temperature of protein and sugar mixtures as measured was always higher than 1°C at a protein/sugar ratio 10/90, whereas the difference increased to 10°C for a mixture ratio 65/35 [49]. Another point that needs to be considered is that freeze-drying below the gassy state is very slow and causes significant operating cost. An increase of only 1°C in product temperature during freeze-drying was found to shorten process time up to 13% [50]. In this case, a state diagram could be used to determine or optimize the freeze-drying process by keeping the sample just below or close to the glass line (i.e. path jmf or lmf) [4].

A step rise in the freeze-drying temperature can be used to control the freeze-drying process using a state diagram [51], and it could lead to an optimum strategy for an instant food powder with desired attributes. Three strategies of freeze-drying (i.e. progressing rise in temperature, and step rise in temperature just above and below the glass line) are shown in Figure 24.10. The initial stage of freeze-drying is performed in the micro-region 9, when frozen water was sublimated, and the drying temperature needs to be below the collapse temperature. This preserves the supra-molecular microstructure. When food passes beyond the maximal freeze concentration condition (i.e. T_m'), unfreezable water is removed by desorption at low pressure (i.e. in micro-region 11). In this drying stage, the temperature should be gradually (or step-wise) increased by following the glass transition line (i.e. just above EQ) in order to accelerate dehydration (Figure 24.2). In this way, the matrix is maintained in a viscosity range for which water diffusion is favored and crystal growth is still slow. Avoiding sucrose crystallization during the desorption step (i.e. micro-region 11) of the unfrozen water in the concentrated matrix enhanced sweetness perception since the amorphous sucrose coating dissolved faster in an aqueous solution as compared to crystalline one. Consequently, this approach of the smart use of the state diagram could guide the development of reduced-sugar food products [52].

24.3.3 Baking

The four stages of the baking process can be visualized and their structural characteristics could be explained using a

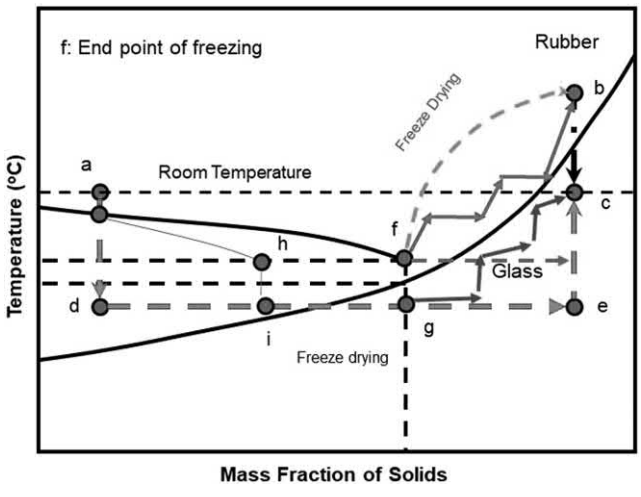

FIGURE 24.10 Freezing-drying strategies with progressive rise to the final freeze-drying temperature (line fb), drying just above the glass line (point f to b with step rise in drying temperature), and drying just below the glass line (point f to c with step rise in drying temperature).

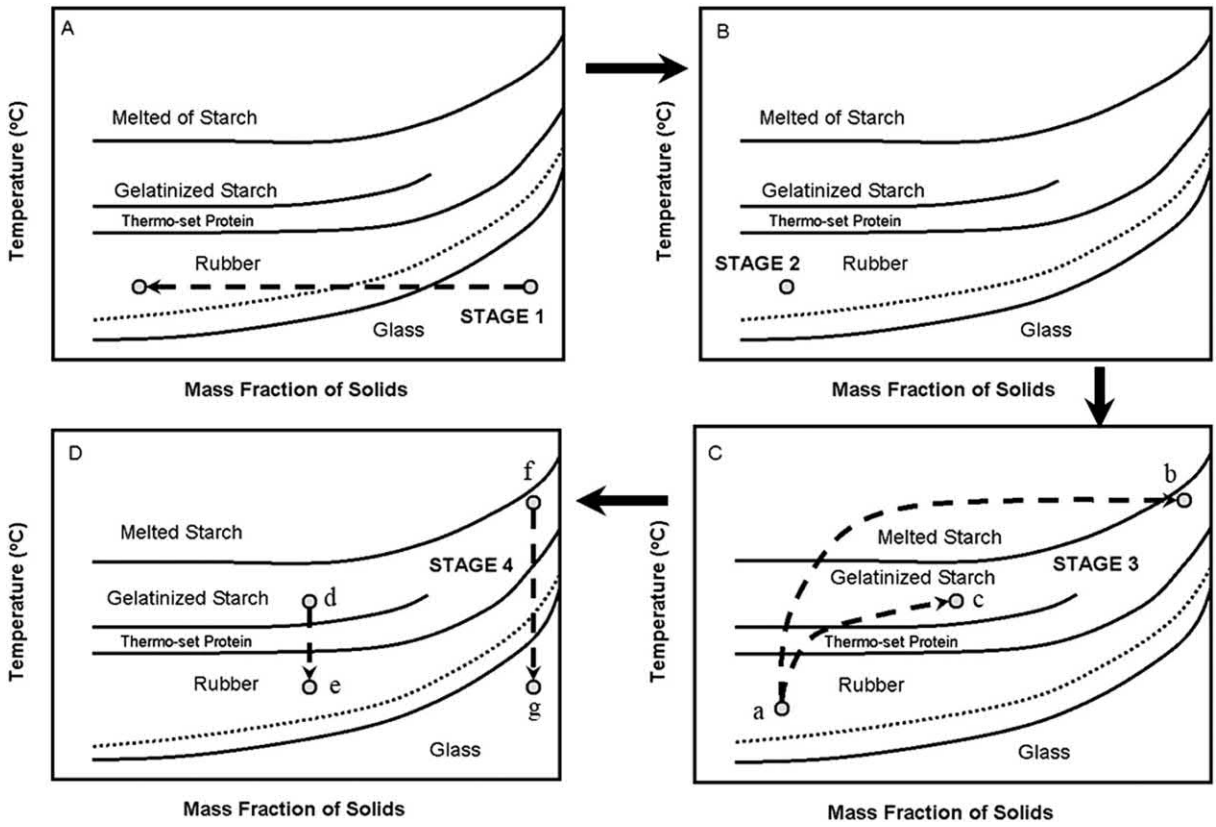

FIGURE 24.11 Paths of the baking process in the state diagram, A: STAGE 1, B: STAGE 2, C: STAGE 3, D: STAGE 4. (From Rahman [4].)

state diagram [3, 4, 53]. Figure 24.11 shows the glass lines of starch and gluten protein, the gelatinized line, the thermosetting line of protein, and the solids-melting line of starch. The glass transition range of proteins appeared to be at slightly lower temperatures than for wheat starch. The drying ingredient mixture is at the state marked STAGE 1 (Figure 24.11A). The second step is associated with a water–flour mixing process until the final water content reaches a level so that it can hold the expanded bubbles (for example, moisture 0.78 g/g sample) (i.e. STAGE 2, Figure 24.11B). This stage is the beginning of kneading and continues until the flour particles are fully hydrated. Flour particle hydration is a very important stage because the wheat gluten proteins pass their glassy state, indicating an increase in protein molecular chain mobility. Protein mobility alone is not sufficient for proteins to interact with other components to form a continuous structure. Hydration also needs to occur in starch, which causes swelling of starch granules and transformation of amorphous molecular organizations above glass transition. In this stage, simultaneous hydration and mechanical kneading induce interactions between proteins and starch, and lead to a rubbery state by the continuous interlinked viscoelastic structure of dough. In this stage fermentation continues, and rubbery dough can hold the gas bubbles like a balloon without collapsing.

In the third stage (STAGE 3, Figure 24.11C), the baking process continues, and dry crust is formed through the path a to b and crumb follows through path a to c. The dough surface is exposed to high temperatures (i.e. 200°C) and substantially dehydrated with irreversible reactions, such as dextrinization, caramelization, non-enzymatic browning, and thermal degradation. These reactions result in the formation of colored and aromatic substances, which characterize fresh bread.

The crust restricts the water loss from dough and forms bread crumbs by gelatinization of the starch and gluten network as a thermosetting rubbery state. The final stage (STAGE 4, Figure 24.11D) is obtained after baked product is cooled (i.e. crumbs d to e and crust f to g) and the crumb (e.g. moisture: 0.35–0.40 g/g sample) forms rubbery and the crust (e.g. moisture: 0.03–0.07 g/g sample) forms glassy states. Consequently, structural hardening occurs and is responsible for crust crispness, while the smooth texture of crumb is due to the rubbery state.

24.3.3 Enzyme Stability as Affected by Crystallization

The enzyme stability was analyzed in a frozen matrix (marked as A in Figure 24.12) and a dehydrated matrix (marked as B in Figure 24.12) in relation to the sugar (dried systems) or water (frozen systems) crystallization [54]. Honey (T_g': –46°C) and trehalose (T_g': –44°C) as solids were considered, and these are stored as frozen systems at –26°C, while the dehydrated systems were stored at 55°C. It was observed that the amount of ice formed also affected the stability in addition to T_g'''. The

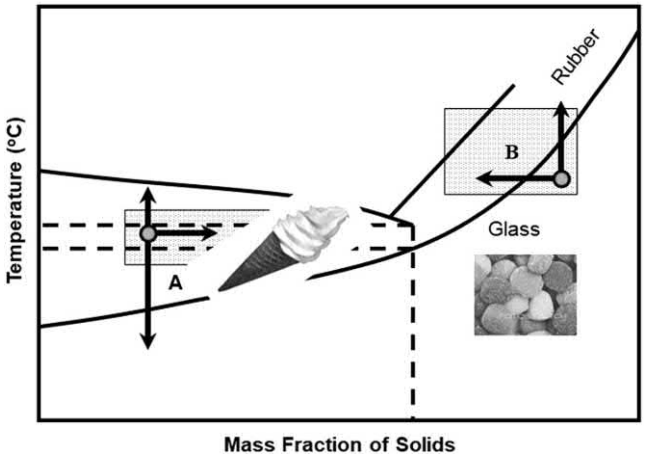

FIGURE 24.12 Crystallization or re-crystallization process in the samples containing freezable and unfreezable water, A: frozen state, B: dried state.

amount of ice may surround the reacting aqueous phases, thus reducing the reactivity of the system. It was also observed that the salts could have positive or negative effects (depending on the types of matrix) both in frozen (region A) and dried systems (region B) although the addition of salts lowered the T_g or T_g''' values. The negative effect of salt due to crystallization could be explained by the change in pH and/or ionic strength. Therefore, a state diagram could be the starting point for analyzing the stability of biomolecules with complementary knowledge of molecular interactions within the matrix [4, 54].

24.3.4 Rice Quality during Drying and Tempering

The drying process can be visualized as a state diagram (Figure 24.13) [4, 55]. The drying process of rice could start from point 1 (either glassy or rubbery depending on the moisture contents) and reach point 2. The drying process occurs in the rubbery state. However, a gradient (moisture or temperature) could exist within the kernel. The rice kernel could have a rubbery core and glassy surface based on the drying

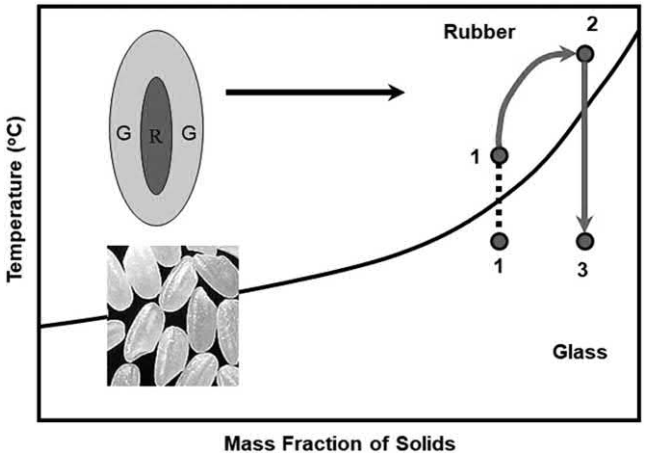

FIGURE 24.13 Air-drying path of rice in the state diagram. (From Rahman [4].)

conditions used, and this can create a differential thermal expansion coefficient. This process may create stresses within the kernel, and if greater than the kernel's tolerated limit, this stress could cause the kernel to fissure (i.e. quality loss) [55, 56]. After the completion of drying, the rice kernel reaches point 3 during cooling. This mechanical stress could be avoided or reduced if all regions in the rice are maintained at the rubbery state. This could be achieved using a drying temperature much higher than the glass line and keeping higher relative humidity of the drying air (i.e. ability to keep the surface rubbery rather glassy). This approach could reduce the fissure. Overall slower drying did not cause sufficient moisture gradients. Alternatively, we could run the complete drying process in the glass state (i.e. path 1 to 3); however drying rates from path 1 to 2 would be very fast as compared to path 1 to 3, due to the very high water diffusivity in the rubbery state (i.e. path 1 to 2). The consequence is the high processing cost for longer operation.

Another alternative is to use an annealing step. Cnossen et al. [56] proposed two options (one in the rubbery state, i.e. point A, and another one in the glassy state, i.e. point B) for tempering as shown in Figure 24.14. Cnossen et al. [57]) showed experimentally that drying and tempering at location A above glass transition (i.e. 60°C) tempering reduced the number of fissured rice kernels. However, drying at location B below glass transition (i.e. 40°C) did not require tempering, and even tempering at 40°C did not decrease fissured kernels further. This example indicates that a state diagram could guide the identification of the location of tempering in order to avoid rice fissure.

24.4 EMPIRICAL APPROACH TO COMBINE WATER ACTIVITY AND GLASS TRANSITION CONCEPTS

An attempt was made to explain the browning of banana as a function of water content and storage temperature by combining the glass transition and water activity concepts [9]. At a specific moisture content, the reaction rate constant showed a shift (i.e. sample containing freezable water) or change in slope (i.e. sample containing unfreezable water), when plotted as a function of temperature (Figure 24.15). However, it was difficult to find any validity above or below glass transition (or BET-monolayer) when all data points (i.e. all moisture and temperature) were plotted (i.e. rate constant with moisture or temperature). An Arrhenius plot at moisture content 0.04 g/g sample showed two linear regions (i.e. below and above critical temperature 45°C) with activation energy values of 105.3 and 25.1 kJ/mol, respectively. Two dimensionless terms, X_w/X_{wb} and T/T_g were defined based on water activity and glass transition. Figure 24.15 shows the plot of rate constant (s^{-1}) as a function of X_w/X_{wb} and T/T_g (i.e. for samples containing unfreezable water) or T/T_g''' (i.e. for samples containing freezable water), respectively. The scattered points of these plots showed that there was no pattern below or above the critical limits of X_w/X_{wb} =1 and T/T_g or T/T_g''' =1, when both moisture

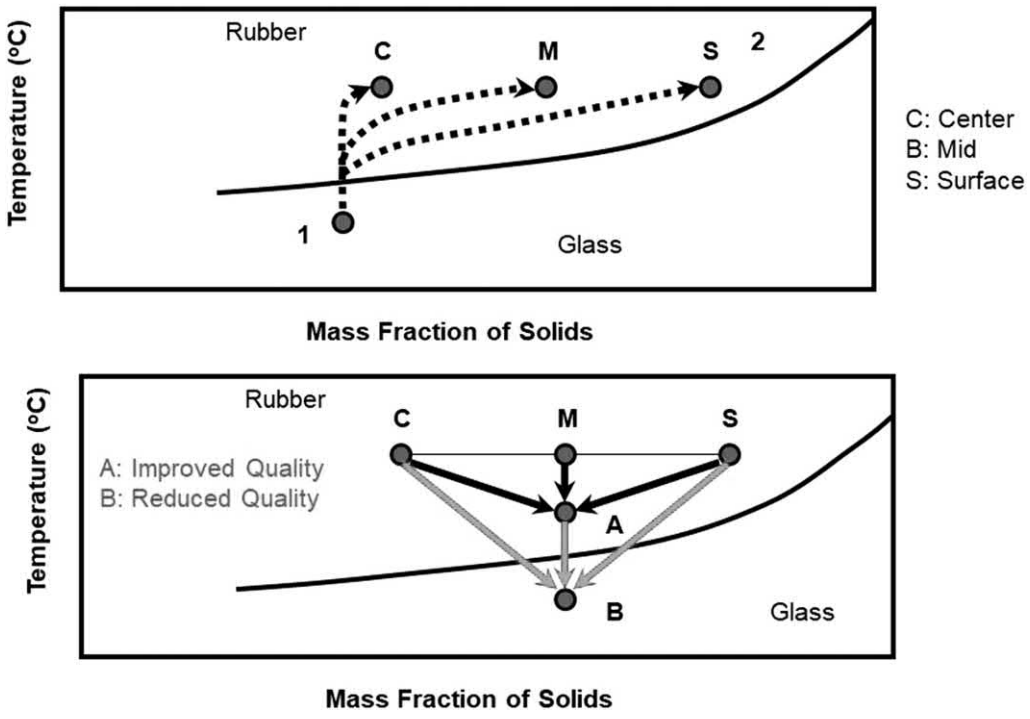

FIGURE 24.14 Rice-tempering strategy using state diagram. (From Rahman [4].)

and temperature changed in combination rather keeping moisture content fixed and only temperature varying or *vice versa*. This indicated that the combination of water and temperature interacted differently at each point, and other structural and physicochemical factors also played a role instead of only glass–rubber or BET-monolayer concepts based on water content.

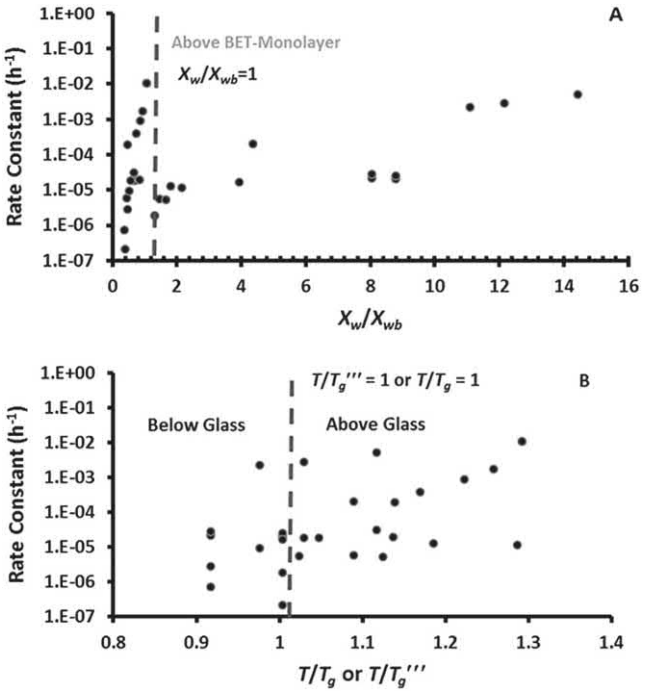

FIGURE 24.15 A: plot of log k as a function of X_w/X_{wb}, B: plot of log k as a function of T/T_g or T/T_g'''. (From Rahman and Al-Saidi [9].)

It was observed that the rate constant showed much higher correlation with T/T_g as compared to X_w/X_{wb} when the whole spectrum of moisture content and temperature was considered (Figure 24.15). This is expected since glass transition could consider the effects of both temperature and moisture contents to some extent, but water activity could handle mainly the effect of moisture (i.e. minimal effect of isotherm with temperature). Rahman and Al-Saidi [9] developed an empirical correlation as ($p < 0.001$ and $r^2 = 0.631$):

$$\ln k = -30.874 + 0.417\left(\frac{X_w}{X_{wb}}\right) + 18.123\left(\frac{T}{T_g}\right) \quad (24.11)$$

where X_{wb} is the BET-monolayer in weight basis (g/g sample), T_g is considered if the sample contains unfreezable water, otherwise T_g''' needs to be used if the sample contains freezable water, and temperature used in the above equation is in K. The individual r^2 for using only terms 1 or 2 was shown to be 0.144 and 0.295, respectively, while the combination provided $r^2 = 0.631$. Figure 24.16 shows a plot of the predicted line with the experimental points. Therefore, the prediction could be further improved if other hurdles, such as pH and compositions, are included in the model. These variabilities indicated that other factors, such as pH, reduction potential, and structure forming or breaking in the matrix affected the reaction rate constant. However, this model could not be considered as generic and unified until other factors, such as different types of reaction, varied matrices, and other hurdles, are included in the model.

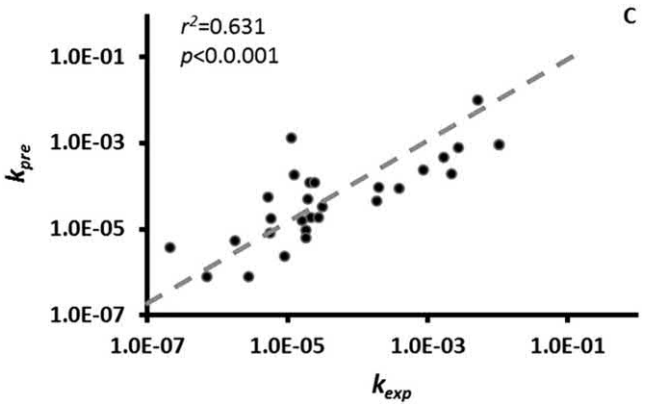

FIGURE 24.16 Plot of predicted (k_{pre}) and measured (k_{exp}) rate constants. (From Rahman and Al-Saidi [9].)

24.5 CONCLUSION

The micro-region state diagram could be a powerful tool in determining food stability during processing and their stability during storage. Although an initial state diagram was developed considering only the freezing curve, glass line, and maximal-freeze-concentration condition, currently different state or phase lines are being included for example, the BET-monolayer line, solids-melting line, soluble solids crystal-melting line, and eutectic point. Currently the micro-region state diagram could be very useful for determining different state or phase boundaries of foods when these are exposed to different temperatures and moisture content.

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25 Concentration Using Membranes

Shyam S. Sablani

CONTENTS

25.1 INTRODUCTION

Separation processes based on membrane utilize semipermeable membranes of the appropriate physical and chemical nature to separate molecules primarily on the basis of size and to a lesser extent on shape and chemical composition [1]. In these processes, the membrane acts as a selective barrier, enriching certain components in a feed stream and depleting it of others. Reid and Breton [2] who used cellulose acetate membranes for desalination of water made the first real breakthrough. Shortly thereafter, Loeb and Sourirajan [3, 4] developed the casting procedure for asymmetric cellulose acetate membranes. The most attractive feature of the process is its simplicity. It involves only bulk movement of fluids using mechanical energy (i.e., pumping). Membrane concentration processes have several advantages over conventional concentration processes, i.e., evaporation. Undesirable heat-related changes such as color, aroma, and viscosity characteristics are avoided because membrane processes can be operated at room temperature. Unlike evaporation or freeze concentration, membrane separation does not involve a phase change for separation; thereby energy is used more efficiently.

Membrane processing in the food industry has been applied mainly for the clarification of fruit juices using microfiltration and ultrafiltration, and for concentration of fruit juices and dairy products. Clarified fruit juices may have better quality and stability, while concentrated beverages are desirable for transport and storage. Filtration rate and product quality are influenced by pretreatment of product, selection of membrane system, and operating parameters. This chapter presents the principle of membrane separation processes, membrane materials and modules, performance measurement of the membrane system, and application of membranes for selected food groups.

25.2 PRINCIPLES OF MEMBRANE SEPARATION

Membrane processes include a wide range of unit operations from sieving to reverse osmosis. Filtration of coarse particles, i.e., in the micron range, is carried out by conventional dead-end filtration where particles are retained by the filter and

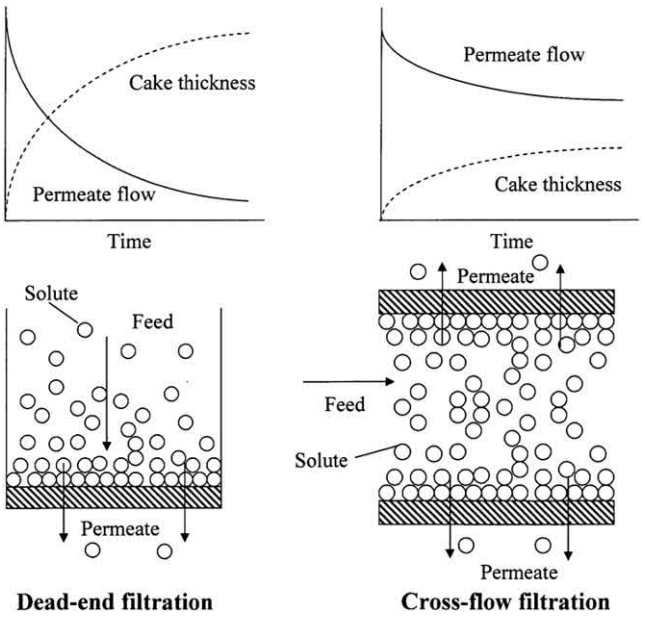

FIGURE 25.1 Dead-end and cross-flow filtration. (a) Dead-end filtration. (b) Gas-flow filtration. (Adapted from Mannapperuma [5].)

later form a cake layer, resulting in increased resistance to filtration. This requires frequent cleaning and replacement of filters. The most common membrane configuration used in the industrial setup is cross-flow membrane filtration. It is a continuous type and used to separate particles, which are about 10 μm to solute molecules that are a few angstroms [5]. In cross-flow membrane separation, the bulk phase is forced to flow along the membrane surface using external pressure. The permeate (less particle concentration) is collected on the low-pressure side of the membrane, while on the high-pressure side the concentrate sweeps the retained particles so that the cake layer remains relatively thin and the resistance to filtration remains low (Figure 25.1). The flow of the liquid through the membrane is driven by the hydraulic pressure gradient. while flow of the solute through membrane is by diffusion driven by concentration gradient [5].

The membrane filtration is divided into four narrower ranges based on particle size: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) (Table 25.1). The size of particles retained in these processes range from 0.1 to 10 μm (microfiltration), 1000 to 500,000 molecular weight cut-off (ultrafiltration), and 100 to 1000 molecular weight cut-off (nanofiltration). The reverse osmosis membranes can retain the smallest solute molecules, such as sodium chloride, and these are classified by percentages rejection of sodium chloride in an aqueous solution under specified conditions and ranges from 95% to 99.5%. The operating pressure ranges are 10 to 50 psi (microfiltration), 20 to 200 psi (ultrafiltration), 100 to 500 psi (nanofiltration), and 200 to 1500 psi (reverse osmosis). Mechanisms of membrane transport proposed are sieve mechanism, hydrogen-bonding mechanisms, solution-diffusion mechanism, and preferential sorption-capillary flow mechanism [1].

25.3 MEMBRANE MODULES

Materials are used for the manufacturing of membranes including sintered metals, ceramics, and polymers (Table 25.2). The membrane structure varies in its chemical nature, microcrystalline structure, pore size and pore size distribution, and degree of asymmetry. Two simple parameters, membrane permeate flux and solute rejection, are used to describe the characteristics of membranes. Since the properties of membrane material can be influenced by environmental conditions and time, secondary properties such as resistance to compaction, temperature and chemical stability, and resistance to microbial attack are also important. Additional requirements for food processing are good tolerance of cleaning and disinfecting solutions, and lack of toxicity of the contact materials [1].

Membranes are assembled as modules that are easily integrated into systems containing hydraulic components. The modules are designed to contain a large membrane area in a small volume, to withstand the pressures required during separation, and cross-flow velocities required to maintain

TABLE 25.1
Membrane Filtration Range

Filtration Spectrum	Diameter of Pores in Membrane (Micron)	Molecular Weight (of Solute)	Filtrate
Microfiltration	0.05–5.0	> 1,000,000	Latex, blood, paint pigment, indigo dye, yeast, bacteria, plant gums, amylopectin
Ultrafiltration	0.005–0.1	4000–10,000	Colloidal silica, virus, enzymes, protein, gelatin, amylose
Nanofiltration	0.0005–0.01	100–5000	Synthetic dye, antibiotics, colorant, amino acids, sugars
Reverse osmosis	0.0001–0.001	<800	Atoms, metal ions, fragrance, flavors, salts

Source: Adapted from Mannapperuma [5].

TABLE 25.2
Polymer, Ceramic, and Metallic Base Membranes and Their Filtration Range and Modules

Membrane Material	Filtration Range	Module
Polymers		
Cellulose acetate (CA)	NF, RO	FP, TU, HFF, SW
Polyamide (PA)	NF, RO	FP, TU, HFF, SW
Sulfonated polysulfone	UF, NF, RO	FP, TU, HFF, SW
Polysulfone	MF, UF	FP, TU, SW
Polyethersulfone	MF, UF	FP, SW
Polyvinylidene fluoride	MF, UF	FP, TU, HF, SW
Polytetrafluoroethylene	MF	FP, TU, SW
Polypropylene	MF	FP, HF
Polyacrylonitrile	MF, UF	FP, HF, SW
Polycarbonate	MF	
Polyester	MF	
Ceramics/Metallic		
Alumina	MF	TU
Zirconia/alumina	MF, UF	TU
Zirconia/metal	MF	TU
Zirconia/carbon	MF	TU
Silica	MF	TU
Silicon carbide	MF	TU
Titanium oxide/metal	MF	TU
Sintered steel	MF	FP, TU
Sintered alloys	MF	FP, TU

Source: Adapted from Mannapperuma [5].

Note: MF, microfiltration; UF, ultrafiltration; NF, nanofiltration; RO, reverse osmosis; FP, flat plate; TU, tubular; HF, hollow fiber; HFF, hollow fine fiber; SW, spiral wound.

a clean membrane surface [5]. The most common module configurations are flat plate, tubular, hollow fiber, and spiral wound (Figure 25.2). Each design has its own advantages and disadvantages.

In flat plate modules, two flat sheets are separated by a support plate that also contains the permeate channels. These membrane sandwiches are separated by a spacer, which also has feed-flow channels. Alternate layers of membrane sandwiches and spacers are assembled and held together by bolts. Advantages of such a system are (i) fairly low holdup and moderately high packing density, (ii) easy membrane replacement, (iii) flexible with regard to membrane usage, (iv) simple to increase the capacity, and (iv) the module can withstand high pressures. The main disadvantages are the system is susceptible to fouling by suspended particles and high initial capital cost. Tubular modules consist of membrane cast inside a porous support tube, typically 6 to 25 mm in diameter. Several such tubes are housed within one pressure vessel in a shell and tube arrangement. Advantages of tubular modules include (i) high turbulence, (ii) ability to handle suspended particles of 1 to 1.5 mm; and (iii) easy cleaning. The major disadvantages are low surface area to volume and high energy costs for pumping.

Hollow fiber modules consist of hollow fibers typically 0.5 to 3 mm in diameter sealed into a plastic header and assembled in permeate castings. The feed passes through the central bore and permeate collects in the outer casing. These can accommodate moderate levels of suspended particles and can withstand low pressures. Hollow fine fiber modules are made with strands of fine fiber about 50 to 100 μm in diameter. A bundle of fibers is formed into a U-shape and the ends are formed into a single header, and U bundle is placed in a tube. The feed liquid is outside the fibers while the permeate flows into the fibers. Some advantages of this configuration are (i) compact, very high packing density; (ii) relatively low holdup; and (iii) high resistance to compression hence it can withstand high pressures. Some disadvantages are (i) extremely susceptible to fouling by suspended particles, (ii) difficult to operate in sanitary mode and to clean, and (iii) individual membrane elements (i.e., fibers) cannot be replaced when damaged. Spiral wound modules utilize flat sheet membranes. Two membrane sheets are sandwiched with a permeate spacer between them and three edges are sealed. The fourth edge is connected to a central perforated tube. A feed channel spacer is placed on top of one layer, and the membrane-screen composite is rolled into a spiral configuration around the central collection tube. The module is placed inside the tubular pressure vessel. Feed flows longitudinally in the feed channel, while permeate flows between the membrane sandwich and spirally around to the permeate collection tube [1]. The advantages of such systems are (i) relatively high packing density, (ii) low cost per unit membrane area, (iii) easy replacement of modules from the pressure vessels, and (iv) low energy consumption. Some disadvantages are difficulty in cleaning when fouled with large amount of suspended matter and prefiltration is needed. The selection of module configuration and membrane material depends on the feed type and economics.

25.4 PERFORMANCE OF MEMBRANE SEPARATION SYSTEMS

The performance of membrane separation systems is calculated in terms of permeate flux and solute rejection. Permeate flux is defined as the volume of permeate produced in a unit time period through a unit area of membrane:

$$\text{Permeate Flux} = \frac{\text{Permeate volume}}{\text{Membrane area} \times \text{Time}}$$

It is measured as liters per square meter per hour. It indicates the ability of the module to produce volumes of filtrate in a given time. Solute rejection is defined as the solute retained by the membrane as a fraction of the solute in the original feed stream and it is expressed as

$$\text{Solute rejection} = \left[1 - \frac{\text{Solute concentration in permeate}}{\text{Solute concentration in feed}}\right] \times 100$$

Solute rejection indicates the ability of a membrane to produce a degree of purity of permeate with respect to the solute concentration. The major objective in the design and selection

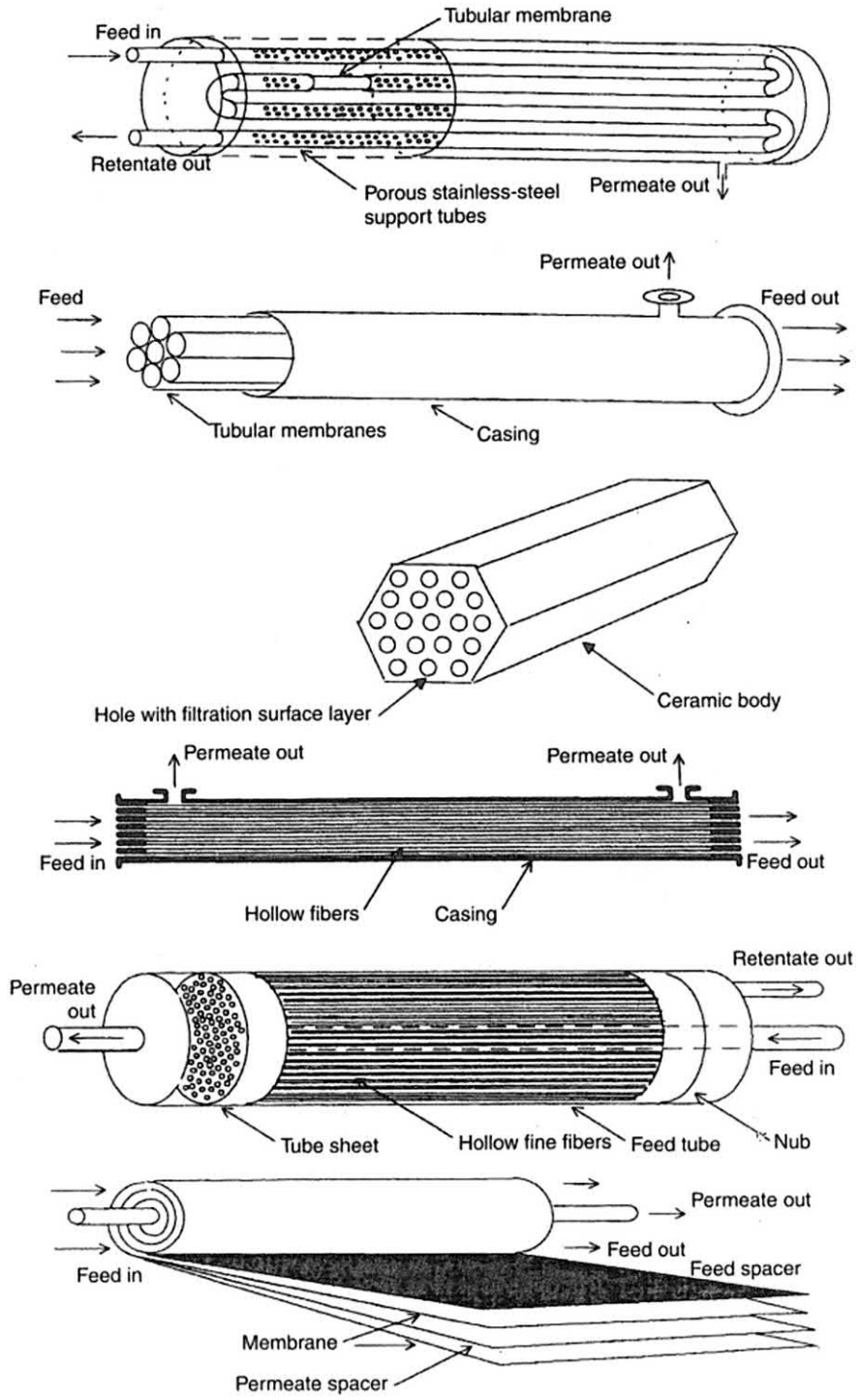

FIGURE 25.2 Membrane modules. (Adapted from Mannapperuma [5].)

of a membrane module for a given feed is to maximize permeate flux and solute rejection.

Permeate flux and solute rejection depends on operating conditions during the filtration process (Figure 25.3). Permeate flux increases with feed pressure, but the osmotic pressure at the membrane surface also increases due to the concentration polarization of the solute. There is a moderate increase in solute rejection with the increase in feed pressure. The feed temperature increases the permeate flux due to decrease in viscosity of feed and change in membrane structure. The concentration of solute in the feed decreases the permeate flux due to the increase in osmotic pressure. The solute rejection also decreases due to the increase in solute concentration in the feed. The increasing cross-flow velocity of feed increases the permeate flux due to the enhanced mass transfer coefficient, which increases with the increase in cross-flow velocity. The solute rejection increases with the increase in velocity and is similar to its variation with pressure.

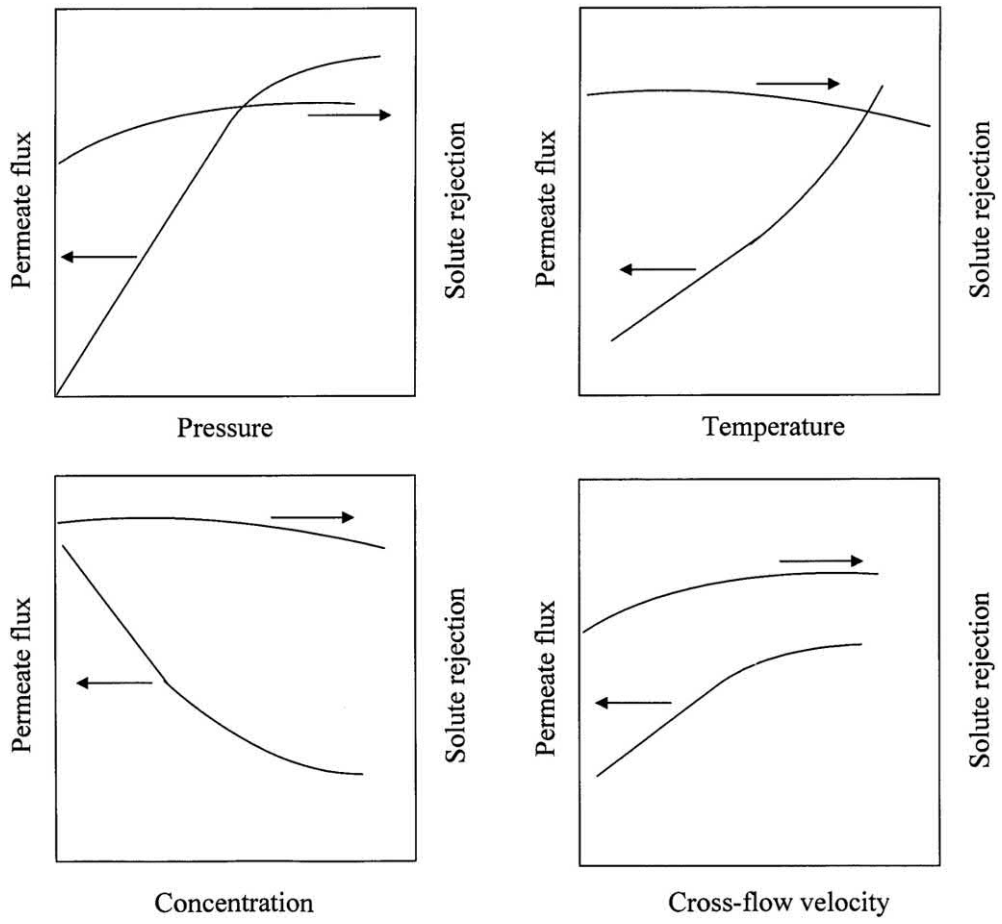

FIGURE 25.3 Performance characteristics of reverse osmosis. (Adapted from Mannapperuma [5].)

25.5 APPLICATIONS IN THE FOOD INDUSTRY

In recent years, the application of membranes in the food industry has increased tremendously. The application ranges from the use of microfiltration to reverse osmosis membranes. In terms of products, it includes fruit juices, dairy, wine and brewery, and fats and oils.

25.5.1 Fruit Juices

25.5.1.1 Clarification

The main application of membrane technology to the fruit juice industry has been for clarification [6]. UF and MF allow the combination of fining and filtration to clarify fruit juices such as apple, pear, cranberry, grape, and citrus (Table 25.3). The main advantages of using membrane technology in the fruit juice industry could be savings in enzymes; short processing time; no addition/removal of fining agents (diatomaceous earth, gelatin, bentonite) required; no problems with wastewater due to fining agents; no over- or underfining, which can result in haze formation; energy savings; no clouding of concentrate; and simple cleaning-in-place. Though juices produced by membrane filtration and traditional methods have mostly been shown to have similar properties, some differences have also been noted. Rao et al. [7] found the retention of odor-active volatile in UF apple juice was intermediate to traditional plate and frame filtration and vacuum drum filtration. Plate and frame filtration gave the highest retention. Rwabahizi and Wrolstad [8] found that strawberry juice clarified through a hollow fiber membrane had an average of 55% anthocyanin loss compared with 17% loss by conventional filtration. Drake and Nelson [9] reported that the UF apple juice had lower turbidity, 5% higher soluble solids, and less color than the traditionally clarified juice.

Pretreatments of fruit juices are important to obtain higher flux and better quality of clarified juice. Depectinization is needed to achieve high flux and concentration factors in membrane clarification processes. Polygalacturonase treatment has been found to decrease the size of granule particles in apple juice as well as to remove their web-like aspect [10]. Reduction in the size of particles and the subsequent decrease in viscosity could improve flux. Depectinization is therefore needed to achieve high flux and concentration factors in membrane clarification processes [6]. Prefiltration and decantation reduce the particulate matter in a juice, which improves flux and allows higher concentration to be achieved. Wucherpfennig et al. [11] reported that the high-molecular weight (>500 kDa) neutral polysaccharides in sloe juice and

TABLE 25.3
Applications in Fruit Juice Clarification

Juice	Membrane Material and Module	MWCO kDa	Operating Parameters*	Flux (L/m²h)	Reference
Apple	Polysulphone, hollow fiber	10–500	25–35°C, 121 kPa	30–275	Padilla and McLellan [100]
Apple	Polysulphone, spiral wound	5	—	28.5	Wu et al. [13]
	Polysulphone, hollow fiber	50	—		
	Ceramic, tubular	0.1 μm	—	111.9	
Apple	Ceramic, tubular	200 A	50°C, 414 kPa	390	Fukumoto et al. [17]
		0.2 μm		190	
	Apple treated with ascorbic acid	200 A		280	
		0.2 μm		280	
Pear	Polysulfone, hollow fiber	10	50°C,	64	Kirk et al. [101]
		30	157 kPa	68	
		50		70	
Kiwi	Polysulfone, hollow fiber	10	20°C, 138 kPa	15.6	Wilson and Burns [102]
Lemon/ orange	Polysulfone, tubular	15–200	20–40°C, 50–400 kPa	10–60	Capannelli et al. [103]; Capannelli et al. [15]
	Polysulfone, hollow fiber	30			
	Polysulfone, plate and frame	30			
	Ceramic, tubular	25			
Tangerine	Polysulfone, plate and frame	25	25°C,	15	Chamchong and Noomhorn [16]
		50	93–194 kPa	34	
		100		45	
		0.1 μm		69	
		0.2 μm		41	

* Temperature and transmembrane pressure.

wine created difficulty in filtration. Citrus juices and tropical juices contain many hydrophobic compounds and clouds that result in lower fluxes.

25.5.1.2 Membrane Selection

The choice of membrane depends on the type of juice and the desired properties of the clarified juice (permeate). The factors that need to be considered while selecting a membrane include its configuration, material, and molecular weight cut-off. Tubular configurations are widely used for fruit juices [12]. They are recommended for clarification because their channels of larger diameters allow for the filtration of feed streams with high solids content and high yields can be obtained. In terms of power consumption per unit permeate of apple juice, Wu et al. [13] found that laboratory systems such as a tubular ceramic MF system (0.1 μm) and a spiral wound polysulfone UF (5 kDa) were comparable (1.15 W-h/L). The total energy requirement for a commercial ultrafiltration process using a two-stage tubular system has been reported to be at approximately 5.3 W-h/L for the production of clarified apple juice.

The choice of membrane material for a given juice is mainly determined by its pressure resistance, temperature resistance, pH resistance, and chemical compatibility. Many materials are suitable under the conditions needed for UF and MF clarification of juices: 1 to 10 bar, 20°C to 55°C, and pH 2.5 to 4. The cleaning protocol for membranes, however, often depends on the choice of membrane material. Other factors to be considered in material selection are its approval for food use, its durability, its availability, and its cost. Polymeric membranes have a life of at least 1 year and ceramic membranes of at least 5 to 7 years under continuous use. However, ceramic membranes are more expensive [6]. Some studies have found that membrane material can have some influence on UF and MF processing. Braddock [14] reported a decline in flux after contact with limonene. Polysulfone membranes had the most severe decline followed by cellulose acetate and Teflon. Rao et al. [7] found that apple juice permeate from polyamide membrane contained more volatile than the polysulfone membrane.

In general, the flux increases with pore size or molecular weight cut-off (MWCO) because the membrane permeability is proportional to the square of the pore radius. For a solute of a given size, retention decreases as pore size increases. Ideally, flux and retention of the haze compound (to be separated) should be as high as possible. The flux always need not increase with pore size [15–17]. Membranes of larger pore size tend to be more susceptible to fouling, as the proportion of smaller particles and colloids in juice increases and can lead to pore blocking and plugging. Juice quality did not appear to be significantly affected by MWCO, although more colored compounds and haze precursors were retained with lower MWCO [6]. The UF and MF membranes have very low

retention of low-molecular-weight compounds such as minerals, acids, vitamins, and sugars. Larger components such as pulp, starch, and pectin show very high retention. Volatiles such as alcohols, aldehydes, and esters in fruits such as apple have low retentions in the retentate [7]. The retention of intermediate weight compounds like enzymes depends on their molecular weights and the pore size of the membrane.

25.5.1.3 Concentration

Several studies have reported the use of membrane processes such reverse osmosis for concentration of a variety of fruit juices, including orange, apple, tomato, pears, grapefruit, kiwi, and passion fruit (Table 25.4). The major constraint with RO is that high concentrations are difficult to obtain due to the high pressures reached by juice retentate. The most important components in fruit juices contributing to osmotic pressure are sugars (hexoses and disaccharides) and organic acids [18]. Table 25.5 lists some osmotic pressures for different juices. The maximum concentration of apple juice by conventional RO is limited to around 30–35°Brix, and the most efficient recovery was 20–25°Brix [19, 20]. With current advances in membrane technology, most juices can be concentrated up to 65°Brix. This can be viewed as a first-stage process with other methods like freeze concentration or evaporation completing the concentration system.

Reverse osmosis has been used commercially to concentrate tomato juice from 4.5°Brix to 8.5°Brix. Later it can be concentrated in evaporators up to 30°Brix. This RO preconcentration can effectively double operating capacity and improve color and flavor characteristics. Similarly, orange juice from 11–12°Brix to 18–20°Brix has been concentrated using RO prior to flash evaporation for producing high-quality concentrate. RO has also been a useful application for maple sap concentration where up to 75% of water can be removed at 1/20 [21].

As in the case of fruit juice clarification using UF and MF, the pretreatment before RO concentration is not always beneficial in terms of flux. It has been reported that both clarified and cloudy pineapple juice had similar flux [22]. However, enzyme treatment may also be useful to assist juice components separation. Invertase catalyzes the hydrolysis of sucrose into glucose and fructose. The use of invertase with pectinase affected a 23% reduction in retained soluble solids and an additional fivefold increase in pigment concentration on a fresh weight basis when processed during RO [23]. Flux of RO is influenced by pressure, temperature, membrane type, flow rate, and concentration [22, 24]. The optimal operating parameters for various fruit juices during RO concentration are reported in Table 25.4. Increasing transmembrane pressure and temperature improve the permeate flux. At higher feed temperature, the membrane permeability coefficient is higher, the diffusivity coefficient in the solution increases, and the viscosity coefficient decreases. The flux has been shown to increase with cross-flow velocity when using RO on liquids containing protein, starches, or other micromolecules.

The membrane material plays an important role in determining the permeation of solutes through the RO membrane due to the interaction of solute and solvent at the membrane surface. Retention of compounds like alcohols and organic acids with a cellulose acetate membrane strongly depends on their polarity or hydrogen bonding capacity. Aromatic polyamide membranes have greater retention of flavor components of apple juice water, but lower fluxes. Recovery of apple juice volatiles during RO concentration to 20°Brix was reported about 80% when using a composite membrane [25].

25.5.2 Dairy

Membrane processes have been investigated in the dairy food industry for concentration of fluid milk and whey. Maubois et al. [26] proposed the use of ultrafiltration of milk before cheese making. The advantages of using ultrafiltration milk were 16–20% increase in the yield of cheese, better control of dry matter and milk fat in the cheese, reduced the quantity of coagulant up to 80%, and reduced environmental pollution due to protein/fat-free permeate/whey.

25.5.2.1 Quality of Ultrafiltered Milk

Milk fat is completely retained in concentrated milk and this increases the yield of cheese due to greater retention of fat in curd than curd made from unfiltered milk [27, 28]. Most of the protein of milk is also retained in the retentate and this increases nitrogenous material in cheese made from UF-treated milk. Calcium in the milk exists in two forms: free ions; and partly bound to casein, phosphate, and citrate. The free ions in the aqueous phase of the milk are not concentrated by the UF. However, the level of calcium-bound casein micelles increases in retentate as the concentration factor increases. The lactose retention in UF milk is very low (~10%). This could cause textural defects and excessive acid production in cheese during ripening if the retentate is used to make cheese. Hence, the level of lactose must be obtained in the cheese curd [27]. The fat-soluble vitamins and water-soluble vitamins, folic acid and B_{12}, bound to protein are completely retained by the UF. Ascorbic acid is mostly destroyed; however, the milk and milk products are not considered as good sources of dietary ascorbic acid [27–29]. The viscosity of concentrated milk is increased drastically due to the increase in the concentration of protein content. This creates difficulty in mixing the coagulant and starter culture in the concentrated milk that may lead to the textural problem in the cheese. Another problem associated with the viscous retentate is entrapment of air that can lead to a spongy-type texture of cheese [30, 31]. The texture abnormalities in the cheese made from homogenized and pasteurized whole milk are fewer than nonhomogenized concentrated milk [32]. The synthesis of curd made from UF milk tends to be slower than by curd from unfiltered milk. The curd obtained by UF milk is relatively difficult to cut, stir, and transport by conventional methods, hence a special coagulator is used [28].

25.5.2.2 Microbiology of UF Milk

The conditions for growth and inhibition of bacteria in UF concentrated milk differ from those of regular milk.

TABLE 25.4
Concentration of Fruit Juices by Reverse Osmosis

Juice	Membrane Material and Module	Operating Parameters	Flux L/m²h	Reference
Orange	Cellulose acetate, flat sheet	2000 psi 10.5–42.0°Brix	0.79–2.83	Merson and Morgan [18]
Maple sap	Cellulose acetate; spiral wound	600 psi 5 gal/min 5.5–10.3°Brix	9.0	Underwood and Willits [21]
Concord grape	Cellulose acetate, flat sheet	1500 psig 0.06–0.55 m/s 15.7–25.3°Brix	0.5–6.2	Lowe et al. [104]
Passion fruit	Cellulose acetate, flat sheet	40 atm 6°C 8L/min 16–26°Brix	1.5–10	Pompei and Rho [105]
Apple juice	Thin film composite, flat sheet	40 bar 30–60°C	9.6 to 21.7	Sheu and Wiley [20]
Apple juice	CA and PA, spiral wound	690–703 psi 21–27°C 13–22°Brix	3–14	Chua et al. [106]
Orange	Plate and frame, spiral configuration	1200 psig 10–12 cycles/min 0.3–0.9 fps 12–14°Brix	2–16	Lowe and Durkee [107]
Apple juice	CA, plate and frame	35–45 bar 20°C 12–25°Brix	8–37	Sheu and Wiley [108]
Beet	CA, plate and frame	30–40 bar 20°C 7–31	12–106	Lee et al.,[23]
Pineapple	Plate and frame, tubular (PCI)	6000 kPa 40°C 13–25°Brix	16–18	Bowden and Isaacs [22]
Orange	Osmonics, 192 MS02-P, spiral wound	6.21 MPa 20°C 15 L/min 10.3–17.8	9–28	Medina and Garcia [109]
Tomato	ZF-99, tubular (PCI)	41.4 bar 40–78°C 4.7–9.5°Brix	18.5–41	Merlo et al. [110]
Tomato	Cellulose acetate, plate and frame	3.0 MPa 20°C 4.8–12.5°Brix	2.3–15.1	Dale et al. [111]
Orange	Cellulose acetate, flat sheet	70–80 kg/cm² 27°C 9.9–46.6°Brix	7–12	Nomura and Hayakawa [25]
Mandarin	Polyacrylamide, flat sheet	711 psi 20°C 5–25°Brix	2–18.5	Fukutani and Ogawa [112]
Grape	Plate and frame	70 atm 6°C 8L/min 10–25°Brix	3.1–14.2	Peri and Pompei [113]
Grape	ZF-99, tubular (PCI)	40 bar 16°C 16.5–22.5°Brix	3.2–13.2	Duitschaever et al. [80]

TABLE 25.5
Osmotic Pressure of Various Fruit Juices at Room Temperature

Fruit Juice	Solids Concentration (%)	Osmotic Pressure (psi)	Reference
Orange	10.5	210	Merson and Morgan [18]
Orange	11.0	230	Cheryan [114]
Apple	15.0	300	Cheryan [114]
Apple	14.0	300	Merson and Morgan [18]
Pineapple	14.0	300	Leightell [115]
Grape	16.0	300	Cherya [114]
Orange	21.5	430	Merson and Morgan [18]
Orange	31.5	850	Merson and Morgan [18]
Orange	42.0	1370	Merson and Morgan [18]
Orange	60	2900	Gostolli et al. [116]

Haggerty and Potter [33] reported that growth and death of *Staphylococcus aureus*, *Streptococcus faecalis*, and *Escherichia coli* in UF-concentrated and unconcentrated milk was not significantly different. Rash and Kosikowski [34] found that *E. coli* serotype 0124 had a greater survival and growth in Camembert cheese made from UF-concentrated milk, and this was attributed to the differences in physicochemical properties of cheese. El-Gazzar et al. [35] reported that *L. monocytogenes* (V7 and California) grew faster and achieved higher counts at 4°C in UF-concentrated milk than in skim milk. Dega et al. [36] previously also reported that heat resistance of several strains of *Salmonella* and *E. coli* increased as the solids content of reconstituted skim milk increased from 10% to 50%.

25.5.2.3 Cheese and Other Dairy Foods

Several studies have been reported on the use of ultrafiltered milk for the production cheese of different varieties. Geilman [37] concentrated skim milk up to 14.5% solids using UF and made cottage cheese of commercial quality. In order to develop a soft coagulum, trisodium citrate was added at the rate of 0.3% of total concentrate. The pH was maintained close to the normal isoelectric point of casein. Lelievre and Lawrence [38] also showed the possibility of making ricotta and cream cheese from UF concentrated milk since these varieties of cheese are not ripened. Olson and Qvist [39] used pasteurized (72–78°C for 15 s) UF concentrate of whole milk to manufacture Feta cheese. Fermentation was carried out using a strain of *S. lactis.* The minimum pH of less than 4.8 was maintained to prevent softening of the cheese during storage. They also indicated that Cast Feta cheese made from UF concentrated milk had favorable characteristics and became popular in Greece. Cast Feta cheese has 3–4% more moisture; a smoother texture; and a strong acid, rancid, and salty flavor. Abd-El-Salam et al. [40] manufactured Domiati cheese using UF concentrated buffalo skim milk (concentration factor 3.5) mixed with fresh cream of 35% milk fat at the rate of 4:1. The Domiati cheese showed less weight loss when stored without salt brine than occurred when cheeses were stored in 5% brine solution or in salted (5%) permeate.

Camembert-type cheeses have successfully been manufactured using UF concentrated milk [29, 41, 42]. For this, skim milk is initially pasteurized and then concentrated about fivefold by ultrafiltration. It was reported that the yield of cheese was 20% higher [41], whey drainage was eliminated [29], and time of production was reduced [42]. Mahaut and Maubois [43] described the manufacturing of blue cheese from UF milk. Cheeses made from concentrate containing 12% protein were as good as or better than traditional blue cheese. Mozzarella cheese can also be manufactured from milk concentrated (two- to fivefold) using ultrafiltration [44, 45]. Colby and brick cheeses were manufactured by Bush et al. [46] using UF skim milk standardized with cream. They reported a reduction in the amount of milk-clotting enzyme used and elimination of curd washing. The brick cheese was more firm and it had a more acid flavor, less typical cheese flavor, and ranked lower in overall preference than did cheese made from regular milk. However, the characteristics of Colby cheese made from UF milk were reasonably close to those of commercial cheeses. Rao and Renner [47] manufactured cheddar cheese from UF milk and reported that the extent of proteolysis was highest in cheese made from unfiltered milk, followed by cheese made from unheated UF concentrate and lowest in the cheese made from heated retentate. There was no significant difference in sensory appearance and constancy among ripened cheese samples. Sharma et al. [48] reported that the cheddar cheese manufactured using UF milk resulted in faster acid development, promoted more proteolysis, caused faster disappearance of lactose, and contributed a smoother body and texture. They used a lower cooking and cheddaring temperature of 35°C. Use of starter culture at 2% by weight of unconcentrated milk together with lowering the cooking and cheddaring temperature caused the pH to be reduced at a faster rate and shortened the cheese-making time by approximately 45 min compared to cheese made using traditional temperature (39°C).

El-Gazzar and Marth [27] concluded from their review that UF concentrated milk can be used for making soft cheese such as Feta, Cast Feta, and Domiati since these cheeses can be consumed without ripening. However, cheese types that require ripening may have problems in sensory properties because of the high content of whey proteins. It has been reported that a significantly slower degradation of casein occurs, and thus cheese from UF milk ripens more slowly than traditional cheese. The higher mineral content in UF-concentrated milk leads to an acid taste and sandy texture in cheese. Acid and bitter flavors also can arise from excessive lactose in the precheese. Also, the behavior of lactic acid bacteria and pathogenic microorganisms differ in retentate from UF milk compared to unfiltered milk. Hence, additional work is needed to solve such problems before it is used more widely in the dairy industry.

The use of reverse osmosis in the dairy industry has been limited. Few studies have reported manufacturing of cheese

from RO-concentrated milk and concentration of whey [49–51]. These studies reported using less starter culture and rennet. However, the major limitation of RO of skim and whole milk has been membrane fouling.

25.5.3 Oils and Fats

Soybean, palm, rapeseed, and sunflower oils are most important in terms of volume of production. Most oils are used for food applications including salad and cooking oils, mayonnaise, margarine, and chocolate. The crude oil extracted from biological material contains fatty-acids, mono-, di- and triglycerides, phosphatides (gums), sterols and tocopherols, and pigments (e.g., chlorophyll). Low concentrations of trace metals, flavonoids, tannins, hydrocarbons, and glycolipids may also be found in some oils. The crude oils need to be refined before they can be sold commercially. This involves removing unwanted components and concentrating the desired products. Usually agricultural oils are extracted using an organic solvent such as hexane, and so the product also needs to be removed from an organic phase. These processes require large amounts of energy and generate large quantities of wastewater. The membrane technology has also potential applications in oil processing. Table 25.6 summarizes some of the research that has been conducted on the use of membrane processing for refining of agricultural fats and oils.

25.5.3.1 Solvent Recovery

Cellulose acetate (CA) RO/NF membranes were used to recover ethanol from cottonseed–ethanol mixtures [52]. The rejection of triglycerides was higher initially. Later, the performance of the system degraded due to alcoholysis of membranes. The polyamide (PA) membranes were also affected by ethanol. Kuk et al. [52] reported that RO/NF membranes with pore sizes 2 nm or less and with a pore density of $10^{12}/cm^2$ were the most appropriate for recovering ethanol from oil. In laboratory tests, Koseoglu et al. [53] found that CA RO and PA RO/NF membranes were most suitable for the separation of *iso*-propanol and ethanol, respectively. However, pilot plant tests showed higher levels of oils in permeate. The increasing temperature in the feed stream also showed poor rejection of oil.

25.5.3.2 Degumming

Sen Gupta [54] found that membranes made with different materials such as PA, polysulphone (PS), polyvinylidene fluoride (PVDF), polyimide (PI), or polyacrylonitrile (PAN) could be used in a UF module for removal of phospholipids from soybean oil in hexane. He reported that phospholipids content of soybean oil was reduced to less than 300mg/L, glycolipids to less than 50mg/L, and green pigments to less than 0.5 mg/L. The concentrations of free fatty acids and tocopherol were unchanged, but the concentrations of copper, iron, magnesium, calcium, and phosphorus were reduced. Sun and Koseoglu [55] found that UF membranes can reduce the

TABLE 25.6
Applications to Agricultural Oil Refining

Membrane Type	Membrane Material	Membrane Module	MWCO kDa	Oil	Process Objective	Reference
RO/NF	PA composite	Flat sheet, tubular	1	Cottonseed	Ethanol recovery	Kuk et al. [52]
RO/NF	PA composite	Flat sheet, tubular	0.5–1.0	Cottonseed	Hexane recovery	Koseoglu et al. [53]
RO/NF	Fluorinated polymer	Flat sheet, tubular	20	Cottonseed	*iso*-Propanol recovery	
RO/NF	PA composite	Flat sheet	0.3–0.4	Cottonseed	Ethanol	
UF	PI	Tubular	20	Soybean	Degumming	Iwama [56, 57]; Miki et al. [117]
UF	PI	Hollow fiber	—	Soybean	Degumming	Sen Gupta [54]
RO/NF	CA	Flat sheet	98% NaCl rejection	Sunflower	Separation	Koike et al. [58]
UF	PAN	Flat sheet	30	Soybean	*iso*-Propanol recovery	Keurentjes et al. [60]
UF	PI	Rotating disk	20	Fish oil hydrolysate	Ethanol recovery	Sahashi et al. [61, 62]
MF	Synthetic polymer (microza TP-313)	Hollow fiber	0.2μm pore size	Sunflower	Dewaxing	Chayamizu and Kikuchi [63]; Watanbe and Chayamizu [64]
UF	Ceramic	Honeycomb monolithic elements (1.9 mm)	0.05–0.2 μm pore size	Soybean	Removal of catalyst	Vavra and Koseoglu [65]
Dialysis membrane	Cellulose	Hollow fiber	6	Soybean	Removal of metals	Keurentjes et al. [59]

phospholipids in cottonseed oil in hexane and *iso*-propane by 98.1% and 70%, respectively. Iwama [56, 57] also reported phospholipid concentrations of 10 to 50 mg/L in permeate of soybean oils in hexane. These values are well within limits acceptable for industrial use. The permeate flux of the hexane-oil mixture varied from 6 to 60 L/(m^2h) at 50°C and a transmembrane pressure of 0.4 MPa.

25.5.3.3 Lipid Separations

Koike et al. [58] found that CA membrane gave the better flux among 18 membranes tested for their ability to separate fatty acids, mono-, di-, and triglycerides from lipase hydrolysate of high oleic sunflower oil. There was a large difference in rejection between free fatty acids and glycerides. The polyvinyl alcohol, PA, and polyether membrane gave high rejections of both glycerides and free fatty acids, but there was little difference between the different components. The permeate flux and rejection were lower in hexane than in the ethanol. Keurentjes et al. [59, 60] found that PAN and cellulose membranes gave complete rejection of triglycerides while separating fatty acids from oil. However, the permeate flux for PAN membrane was 30-fold higher. Sahashi et al. [61, 62] reported a study on the concentration and purification of n-3 polyunsaturated fatty acids (PUFAs). A hydrophilic PI UF membrane was found most suitable for the separation of solvent phase containing free fatty acids as permeate. The permeate flux was in the range of 40–80 liters/(m^2h). Kuk et al. [52] observed differences in rejection between triglycerides of different fatty acid composition when separating cottonseed oil from ethanol using PA RO membrane. They attributed these differences due to variations in viscosity and diffusivity. Tristearin, which had the highest viscosity, was completely rejected, whereas tripalmitin, and mixed triglycerides containing oleic acid, palmitic acid, and lauric acid, were rejected to varying degrees.

25.5.3.4 Dewaxing

In edible oil processing, dewaxing is done between decolorization and deodorization, and is one of the areas where membrane processing has been applied successfully. Asahi Kasei Corporation has carried out membrane dewaxing of sunflower oil on an industrial scale in Japan for more than 15 years. Chayamizu and Kikuchi [63] and Watanabe and Chayamizu [64] reported 97% of wax rejection using a MF membrane of 0.2 μm pore size. Periodic backflushing by gas and with 80°C hot oil was necessary to maintain the permeate flux and to remove any wax that accumulated at the membrane surface. The oil flux was 4.8 to 27 L/(m^2h) under a pressure of 0.3 MPa and the feed oil temperature was 5–10°C.

25.5.3.5 Removal of Contaminants

Keurentjes et al. [59] used a hollow-fiber cellulose membrane module to remove copper from soybean oil. Vavra and Koseoglu [65] and Koseoglu and Vavra [66] used ceramic membranes to separate nickel catalysts from hydrogenated soybean oils. The rejection varied from 82% to 100%. while the permeate flux ranged from 8.5 to 42.5 L/(m^2h). However, the permeate flux with a co-polyalkoxyether-imide (PEI) membrane was 113 L/(m^2h). Kuk et al. [52] and Hron et al. [67] used PA RO/NF membranes to concentrate aflatoxin B1 from cottonseed oil in ethanol or *iso*-propanol.

25.5.3.6 Removal of Pigments

Vegetable oils contain numerous pigments, including chlorophyll, carotenoids, xanthophylls, and their derivatives, that need to be removed to give the oil an acceptable color. Koseoglu et al. [68] identified 5 suitable membranes out of 15 tested for their ability to remove pigments, phosphorous, and gossypol (a yellow pigment) from crude cottonseed, soybean, canola, peanut, and meadowfoam seed mixtures. Chlorophyll and β-carotene rejection efficiency varied between membranes and between oils. Typically the color readings of the permeate were about one-tenth that of the crude oils. Diosady et al. [69] suggested the use of a combination of techniques including membranes to reduce chlorophyll by more than 90% from canola oil. Reddy et al. [70] found that PE membrane had a poor rejection of chlorophyll (<4%), but PI composite membrane gave over 95% rejection from sunflower and soybean oils in stirred batch test cells.

25.5.3.7 Membrane Bioreactors

In recent years there has been lots of interest in the enzymatic modification of fats and oils to produce high-value products from cheap and plentiful raw agricultural materials. The process involves the use of lipase to catalyze hydrolysis, ester synthesis, and interesterification reactions. In the process, membranes can be used to provide a solid-supported interface and recover enzymes for reuse. Many research groups have used membrane bioreactors for the modification of fats and oils. Snape and Nakajima [71] have reviewed the choice of membrane material, membrane characteristics, and reactor configuration.

25.5.4 Potential Applications of Membrane Processes

Pectic enzymes are used in the clarification of apple juice before membrane filtration. The membranes clarification can be used to recover these enzymes to reduce industrial processing costs [72]. The isolation and purification of pectin from fruits can be achieved using ultrafiltration [73, 74]. UF can also be used to recover colloidal carbohydrates that withstand the hydrolytic conditions of commercial pectolytic enzyme preparations during juice clarification [75]. Other major by-products of the fruit juice industry are essential oils and essences. Many of these components are immiscible with and poorly soluble in water, hence making UF and RO potential recovery techniques. Membrane processing techniques have been investigated for the purification and concentration of various pigments such as anthocyanins and betanins [23, 76, 77].

The membrane processes can be combined with ion exchange to deacidify fruit juices. Snir et al. [78] used ultrafiltration permeate of passion fruit juice and deacidified using ion-exchange chromatography and obtained a less sour

product with a flavor similar to the original juice. Koseoglu et al. [79] treated the UF permeate of citrus juice with RO and ion exchange for deacidification, while UF retentate was heat sterilized. The treated permeate was combined with sterilized retentate to obtain better flavor and aromas.

25.5.4.1 Wine

Reverse osmosis has been investigated for concentration and dealcoholization of wines [6]. Wines of low alcohol are difficult to preserve and market. In order to increase the sugar concentration in these wines, reverse osmosis has been applied as an alternative method to evaporation and freeze concentration. Duitschaever et al. [80] used thin film composite membranes in a plate frame module to increase sugars by 2.4%. Spiral wound and hollow fiber membranes have also been used for partial concentration and grape musts clarification prior to RO using centrifugation or microfiltration [81–83]. The initial must from immature grapes can be concentrated with excessive malic acid content and reinforce the vegetal character using RO [84]. A low-acid, light-colored, and light-bodied red wine can be concentrated using RO to produce a full-bodied, dark-colored, high-acid product. Dick and Dixmier [85] used polyhydroxymethylacrylamide RO membrane with 85% to 90% ethanol rejection increases ethanol concentration from 10% to 12.5%, while the alcohol content in the permeate remained below 2%. Most other constituents were found at trace levels in the permeate except methanol, which can cross membrane freely [85, 86].

Selective RO membranes have been used to dealcoholize wines. The process involves wine as a feed and the RO membranes allow water and ethanol to permeate, while most flavor and all color compounds are retained. The wine is recycled across the membranes until the required degree of concentration is achieved. The permeate containing water and ethanol is distilled and water is combined with concentrated wine to produce a low alcohol product [6]. It is feasible to produce wine with 3% ethanol with acceptable quality [87]. The sensory properties of dealcoholized wine started to change significantly below 9% of alcohol. The wine appears to have less body and a more watery mouthfeel. In order to have acceptable products, organic acid and grape juice/concentrate are added to wines to obtain 6 g/L titratable acidity and 5°Brix [88]. Wucherpfennig et al. [89] used the dialysis process for the dealcoholization of wine using another alcohol-free wine produced using vacuum distillation. As a concentration gradient exists only for alcohol, little change in concentration of other constituents occurs. The alcohol diffusing into the second wine is eliminated by vacuum distillation, allowing reuse of the wine in the dialysis process. This way, heat damage to the wine is eliminated. Bui et al. [90] used two reverse osmosis modules, one equipped with ethanol permeable polysulfone membranes and the second with selective ethanol retention polyacrylamide membrane. The ethanol retention membrane produced concentrated wine (12–13% ethanol) and permeate (mainly water and 2% ethanol) is mixed with original wine (10–11% ethanol) and used as feed to ethanol permeable membranes to produce light wine with 6–7% ethanol. Because membranes were impermeable to anthocyanins, two different wines, such as rose and red wine, can be used simultaneously for the light and enriched streams [6]. The effects of membrane filtration on the aromatic profile and phenolic quality of Cabernet Sauvignon wine was studied by Arriagada-Carrazana et al. [91]. The results of their study showed that concentration of tannins (4.8%), anthocyanins (2.4%), and total phenolic index (10%) decreased. This was attributed to absorption in the membrane filter.

25.5.4.2 Pervaporation

Mass transport during pervaporation is achieved by partial vaporization through a nonporous selectively permeable membrane. The flux of any compound to be separated is determined by its partial pressure gradient across the membrane and its permeability in the membrane matrix. The difference in partial pressure is created on the permeate side, usually vacuum generated. The process results in a liquid retentate and a vapor permeate that is condensed using low temperatures [6]. One of the first pervaporation applications considered for the food industry was the production of dealcoholized beverages including wine and beer [92, 93]. Hydrophobic membranes can preferentially permeate ethanol over water vapor, and this can be used to extract ethanol from alcoholic beverages. In order to reduce the aroma loss, hydrophilic membranes can be used. Another promising application of pervaporation is aroma compound recovery and concentration. Bengtsson et al. [94] used pervaporation to concentrate a natural aroma condensate from an apple juice concentrate plant. Rajagopalan and Cheryan [95] evaluated pervaporation membranes using a model flavor, i.e., methyl anthranilate compound of Concord grapes. Like other membrane techniques, pervaporation allows a low-temperature extraction and makes it possible to treat heat-labile products, such as beverages or fermentation media. Pervaporation has also been demonstrated as a possible means for juice concentration. Buvet and Idier [96] developed a hydrophilic membrane for extracting water from juices, syrups, and other aqueous solutions. Karlsson and Tragardh [97] investigated the effect of high ethanol concentrations during aroma compound recovery. Though pervaporation has not yet had a major commercial impact in the food industry, it is a very attractive approach to certain separations requiring the avoidance of high mechanical, thermal, or chemical stresses. The potential application of pervaporation is in the aroma recovery in fruit juice processing.

25.6 CONCENTRATION POLARIZATION AND FOULING

The major goal of the membrane system is to achieve required separation with economically acceptable permeate flux. Both concentration polarization and membrane fouling influence the performance of the membrane system (Figure 25.4) [24]. Membrane lifetime and permeate fluxes are primarily affected by the phenomena of concentration polarization (i.e., solute build-up) and fouling (e.g., microbial adhesion, gel layer formation, and solute adhesion) at the membrane surface.

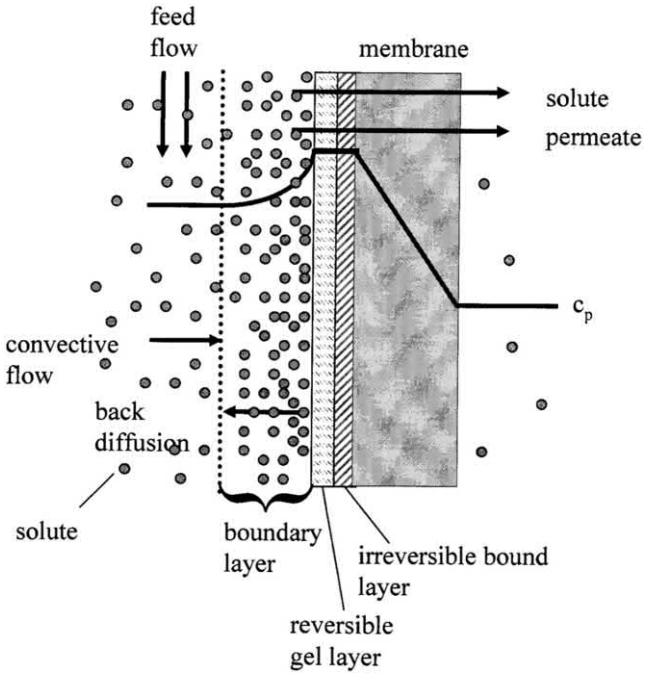

FIGURE 25.4 A schematic representation of concentration polarization and fouling at the membrane surface.

Concentration polarization is a fluid dynamics phenomenon, whereas fouling is a chemical phenomenon between solutes and the membrane. In a laminar flow regime, decreasing channel height, increasing inlet velocity, and decreasing channel length can decrease concentration polarization. Disrupting the boundary layer by mechanically inducing turbulence in the fluid stream can improve permeate flux. The flux can be improved with dynamic turbulence promotion involving acrylic resin plastic spheres moving randomly in the retentate channels of an RO system. The permeate flux can also be improved with reciprocating flow. Depending upon the pulse rate and flow velocity conditions, the permeate flux improved from 1.7 to 5.1 L/m^{2}h for 24% solids orange juice. Backwashing is another technique where the flow is intermittently reversed from feed-side-to-permeate-side flow to wash solutes off the membrane surface and out of the system. Other options such as ultrasonic vibration, rotational motion, and spongy balls have also been investigated [6, 24].

25.7 CLEANING MEMBRANES

Effective cleaning after processing is important to reduce the fouling on the membrane surface. This way flux could be restored and membrane life and performance can be maximized. A wide variety of acid/alkaline–detergent/sanitizer combinations can be applied to remove foulant accumulated in the membrane systems. Membrane manufacturers have spent considerable amounts of effort to identify the appropriate detergent and its strength suitable for particular application. For juice processing, the cleaning involves rinsing the membrane system with water initially, then using alkaline detergent and hypochlorite solutions at recommended temperatures (40–60°C). Typically, a membrane unit used for filtration of apple juice can be cleaned-in-place at 50°C using a 0.1–0.5% alkaline detergent solution (pH 10 to 11) with 100 to 1000 ppm free chlorine, the rates being specific to the type of membrane [98, 99]. Following a rinse with water, the cleaning cycle can be repeated. Mixtures of nitric and phosphoric acid are recommended for dairy processing for dissolving minerals and salt foulant. For most commercial membrane systems, cleaning instructions are provided by the membrane manufacturers. The cleaning protocol includes type of chemicals, time of exposure, and temperature. RO membranes tend to be more susceptible to damage than MF and UF. In general, PVDF membranes can withstand higher rates of chlorine than cellulose acetate or polyethersulfone. It is important to monitor the sanitizer strength and ensure contact with all areas of the membrane system.

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26 Stickiness and Caking in Food Preservation

Bhesh Bhandari and Thao M. Ho

CONTENTS

26.1 INTRODUCTION

Dry solid food materials can exist in various forms and dimensions. This may range from pieces to very fine nanosize powders. The dimensions of individual particulates may vary from a few microns (or even nanosize) to several millimeters or centimeters. Food powders generally have the dimension of few millimeters down to a few micrometers. Granular products (diameters of a few millimeters) are also categorized as powders. In this chapter, dried foods are referred to as powders, since the powder form is the most common dry state of food materials.

Various methods are employed to produce foods in dried form. These processes include various methods of drying, crystallization, grinding, milling, or mixing (liquid to solid) with other ingredients. Depending on the type of process employed and the nature of the components present, the dried product may exist in amorphous, crystalline, or mixed (semicrystalline) form. Some examples of food powders in these states are listed in Table 26.1. The physical form of the powders and their individual properties influence many functional properties such as flowability, bulk density, ease of handling, dust forming, mixing/segregation, compressibility, and surface activity. The stickiness and caking behavior of powders are also related to the physical forms and dimensions of particulates. Fundamental understanding of the sticking and caking behavior of powder is important since these phenomena alter the expected functional and nutritional properties and stability of the food powders.

TABLE 26.1
Physical States of Various Food Powders

Forms	Examples of Powders
Amorphous	Milk, some whey powders, encapsulated powders, instant coffee and tea, spices, cheese, protein, coffee whitener, cocoa, spice mixes (gravy, soup, etc.)
Crystalline	Refined sugar, organic acids, polyols, salts
Mixed	Some whey powders, starch powders, ground icing sugar

Source: Bhandari and Hartel [1].

26.2 STRUCTURE OF FOOD SOLIDS

26.2.1 Crystalline Structure

Many dried solid foods are in crystalline form. Thermodynamically, the crystalline form is at the lowest energy level or stable equilibrium state. The crystalline state has a defined molecular arrangement in the long-range order (Figure 26.1). The molecules in crystalline form are tightly packed; therefore only radical or functional molecular groups on the external surface of the crystals can interact with external materials such as water (absorption). Powders in the crystalline state are the most stable. No caking occurs unless the surface of the crystals dissolves due to a high humidity environment.

26.2.2 Amorphous Structure

The majority of processed dried foods exist in an amorphous form. Thermodynamically the amorphous state is at a higher entropy level than the corresponding crystals. Molecules in this state are nonaligned, tangled, more open, and porous. An amorphous solid may also possess short-range order and regions of high and low densities (Figure 26.1) and have higher entropy than the corresponding crystals. Since an amorphous state is at a high energy level, it can undergo crystallization to achieve equilibrium. Amorphous powders are the most prone to stickiness and caking.

26.2.3 Mixed Structure

Mixed structure dried foods (coexistence of amorphous and crystalline structure) have both amorphous and crystalline regions. The mixed structure occurs during processing, such as partial crystallization or by grinding the crystalline structure. These powders are also prone to stickiness and caking due to the presence of an amorphous structure.

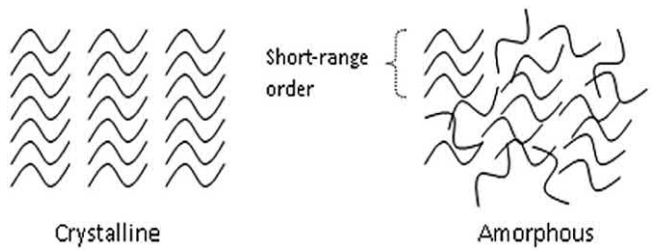

FIGURE 26.1 Schematic representation of crystalline and amorphous molecular structure. (From Bhandari and Hartel [1].)

26.2.4 Characterization of the Structure of Food Solids

The differences in molecular arrangement between amorphous and crystalline solids lead to differences in their physical properties (as shown in Table 26.2) and consequently their stability and functionality in food production. Food solids are very stable in crystalline form, while those existing in an amorphous form are likely to experience various undesirable changes (structural relaxation, stickiness, caking, lumping, agglomerating or crystallization) during handling, storage, and processing. Thus, the presence of an amorphous phase, even a very small amount in food solids, greatly affects the properties and stability of the whole product. Moreover, the production of partial or completely amorphous solids is an intrinsic property of many processes of food production (freeze-, spray-, and drum-drying of a solution, or milling of solid materials). Therefore, characterization of the structure of food solids is essential to determine the further utilization of the product.

Many analytical techniques have been developed to differentiate the structure of food solids, such as X-ray diffraction, microscopic analysis (scanning electron microscope or polarized light microscopy), differential scanning calorimetry, gravimetric vapor sorption, and spectroscopy approaches (Fourier transform infrared spectroscopy, Raman spectroscopy, or ^{13}C nuclear magnetic resonance spectroscopy) [3, 4]. Under these analytical techniques, solid materials with different structures will respond differently. Table 26.3 shows the differences between pure amorphous and crystalline solids analyzed by various techniques. The mixed structure solids can display a combination of properties of completely amorphous and crystalline ones. A typical example of differences among 100% amorphous, 100% crystalline, and mixed structure solid materials under X-ray diffraction (XRD) and differential scanning calorimetry (DSC) is shown in Figure 26.2. In this figure (a) denotes pure crystalline beta-cyclodextrin (β-CD) powder, (b) denotes pure amorphous β-CD powder, and (c) denotes mixed structure complex powder of tea tree oil and β-CD powder [5]. For XRD, there were many sharp peaks for crystalline β-CD powder and three broad peaks were observed for amorphous β-CD, while a combination of the peak shape of pure crystalline and amorphous β-CD powders was witnessed for mixed structure complex powder. Similar results were also found for DSC analyses. The DSC thermogram of crystalline β-CD was depicted by an endothermic peak at 90–140°C, while the amorphous one exhibited an endothermic hump at 40–170°C, and the mixed structure complex powder displayed a combination of the DSC thermograms of completely crystalline and amorphous β-CDs.

TABLE 26.2
Differences in Physical Properties of Amorphous and Crystalline Solids

Characteristics	Amorphous Solids	Crystalline Solids
Structure	• Loosely packed • Unsymmetrical	• Highly packed • Symmetrical
Strength of molecular bonds	Different and small	Equal and large
Heat of fusion	None	Defined
Melting point	Wide range	Extremely sharp
Physical properties	Isotropic	Anisotropic
Scattering produced by incident X-ray	Weak and spread throughout reciprocal space	Strong and concentrated into a few sharp diffraction peaks
Adsorption of external molecules	• Hygroscopic • Adsorption into the bulk of solids	• Nonhygroscopic • Adsorption primarily on the surface of solid particles
Water dissolution ability	High and fast	Low and slow
Compressibility	High	Low
Nature	Pseudo-solids or supercooled liquids	True solids
Clearage property	Irregularly	Defined
True density	Low	High

Source: Ho et al. [2].

TABLE 26.3
Differences between Completely Amorphous and Crystalline Solids under Various Analytical Techniques

Analytical Techniques	Amorphous Solids	Crystalline Solids
X-ray diffraction	One or several broad peaks	Many sharp peaks
Scanning electron microscopy	Spherical shape particles	Irregular shape particles
Polarized light microscopy	Homogenously dark background	Colored bright crystal granules with different shapes
Differential scanning calorimetry	- A big hump or broad peaks representing water (volatiles) evaporation - A signal of structure relaxation, glass transition, and crystallization	- Many sharp peaks representing water (volatiles) evaporation - A signal of melting
Fourier transform infrared spectroscopy Raman spectroscopy	Less defined and high intensity peaks	Sharp or low intensity peaks
Gravimetric vapor sorption	High water adsorption ability and changes of water adsorption behavior due to phase transitions at high relative humidity	Low water adsorption ability and quite stable at high relative humidity
^{13}C Nuclear magnetic resonance spectroscopy	Broad peaks for each carbon atom	Sharp, splitting, or even overlapped peaks for each carbon atom

26.3 STICKINESS OF FOOD SOLIDS

Stickiness of foods is generally an undesirable property. This can cause difficulty in processing, handling, mixing, and storing of food materials. In certain food processing situations, the same property can be useful, such as in agglomeration and coating processes. Stickiness relates to both cohesiveness within the same food material and adhesiveness to different materials. Since stickiness is a surface property, the surface energy of both similar and dissimilar materials plays a role.

26.3.1 Cohesive Forces and Stickiness

Various interparticulate cohesive forces (Figure 26.3) involved in stickiness are (i) liquid bridges, (ii) solid bridges, (iii) van der Waals forces, (iv) electrostatic forces, and (v) mechanical interlocking.

26.3.1.1 Liquid Bridges

Liquid bridges are produced due to melting, wetting, and dissolution of the external surface of the particles or the release of mobile liquid components from the interior of the particles

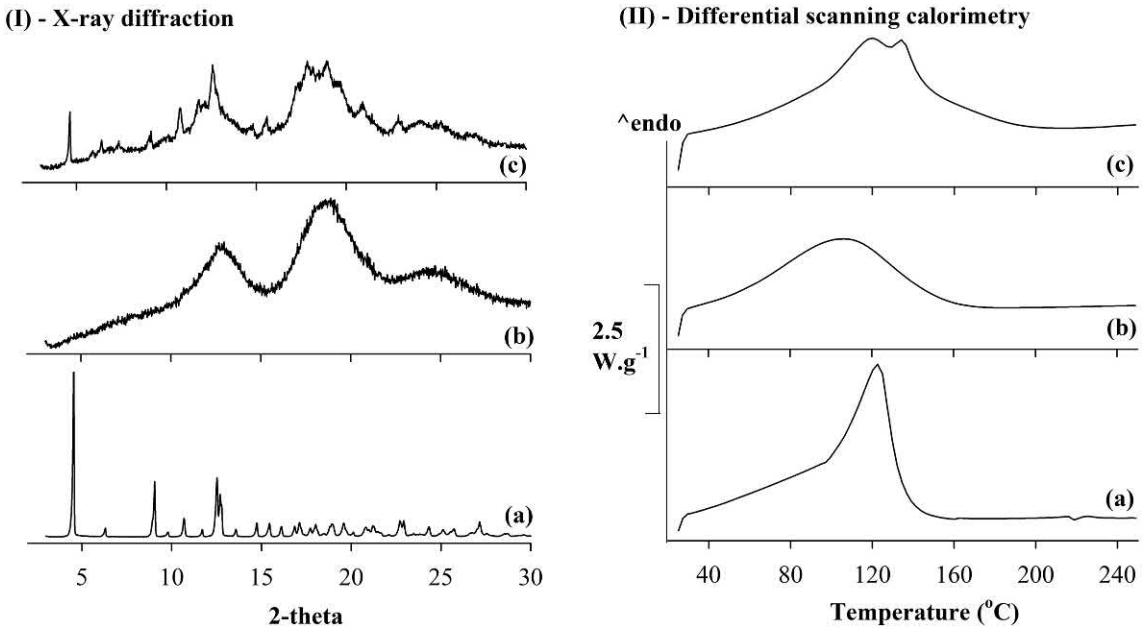

FIGURE 26.2 An example about differences in X-ray diffraction (I) and differential scanning calorimetry (II) analyses among pure amorphous, pure crystalline, and mixed structure. (a) Pure crystalline beta-cyclodextrin (β-CD) powder, (b) pure amorphous β-CD powder, and (c) mixed structure complex powder of tea tree oil and β-CD powder.

(Figure 26.3a). This type of cohesion is mainly dominated by the surface tension and capillary properties. For example, during rewetting of the particles in an agglomeration process there is a flow of liquid between two adjacent particles. Upon removal of solvent (such as water), the mobile liquid bridges turn into solid bridges. However, this type of solid bond can be fragile due to the narrowness of the bridge. The presence of low melting point components (such as oil) also results in liquid bridges. This type of nonaqueous bond is weak due to the noncompatibility with other solids present in the powder. The powder flow is adversely affected by the presence of liquid bridges. Many high-fat powders do not flow well for this reason. Solidification of these liquid bridges due to temperature fluctuation makes the bond stronger.

26.3.1.2 Solid Bridges

Solid bridges between particles are formed by melting and solidifying (sintering) and crystallization of dissolved solids (Figure 26.3b). The interparticulate contact area is large and the strength of the agglomerate is high. Lowering of the temperature of the powder converts the liquid bridges to solid bridges. In some powders, such as milk powders or high sugar powders, this type of bond is so strong that it could need a hammer to break the lump.

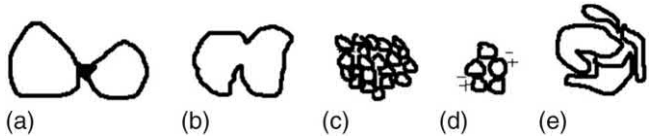

FIGURE 26.3 Schematic diagram of interparticulate cohesive forces. (a) Liquid bridges, (b) solid bridges, (c) van der Waals forces and (d) electrostatic forces, and (e) mechanical interlocking.

26.3.1.3 Van der Waals Forces

All molecules possess weak attraction forces at very close distances. This is due to the electrostatic attraction of the nuclei of one molecule to the electrons of the other. This may result in polarization of the molecules at the surface. Fine powder particles (<1 μm size), which have a very small interparticulate space, tend to stick to each other due to this force (Figure 26.3c). The oscillation of the molecules and vibration of the bonds may also cause such attractions due to facilitation of the alignment of the positive and negative forces. When the force of gravity (e.g., larger particles) is larger than the van der Waals forces, the particles do not show such cohesive behavior. Generally higher molecular weight materials having more electrons tend to be more cohesive. The fundamental mechanism of van der Waals forces is electrostatic in nature [6]. This type of stickiness will be common in submicron or nanoparticles. Deposition of fine powders into the dryer wall, equipment surfaces, and room walls is the result of van der Waals forces. This force is relatively weaker, and therefore can be easily broken. However, due to close proximity and minimum interparticulate space, this force accelerates other types of caking.

26.3.1.4 Electrostatic Forces

There is normally confusion over electrostatic forces and van der Waals forces. The van der Waals is a short-range force, whereas the electrostatic is a long-range force. The fine particles can have some excess electrons due to friction. If these excess electrons are not dissipated (due to low conductivity), the electron-rich particles can realign themselves with electron-poor (oppositely charged) particles to balance the charge (Figure 26.3d). This results in cohesion or adhesion of particles. In fact, van der Waals forces and electrostatic forces

act in combination in the case of cohesion/adhesion of fine powders [6].

26.3.1.5 Mechanical Interlocking

Mechanical interlocking occurs due to the irregular or uneven shape and size of the particles (Figure 26.3e). The fibrous, bulky, and flaky particles interlock with each other or "bird nest" [7]. Under compaction or vibration, particles are repositioned and become more entangled. Upon heating, wetting and drying these physical bonds can become very strong.

The energy of interactions between particles depends on the type of material, moisture content, size, and shape of the particles and external electrical field. In general, the solid bridges are stronger than liquid bridges. The van der Waals forces are the weakest one and their dominance is high when particles are very small and the gravitational effect becomes nominal. The interlocking energy depends on the surface roughness of the particles and the amount of distortion and packing. Barbosa-Cánovas and Juliano [8, 9] compiled some information on the energy of binding of various forces (Table 26.4).

26.3.2 Adhesive Forces and Surface Energetics

As stated earlier, stickiness is related to both cohesion to similar and adhesion to dissimilar surfaces. In the case of adhesion of food particulates onto different surfaces, it is influenced by the adhesive balance between contacting surfaces. In fact, the stickiness property is directly related to the interfacial surface energy of contacting materials. Adhesion of fine particles to dissimilar surfaces is also influenced by their electrostatic charges and the electric conductivity of the contact surface.

In some food processing situations, food adhesion can be of significant concern, particularly in the case of foods that are more adhesive than others. One of the important processes where stickiness has been an issue is the drying of high-sugar and high-fat products [10, 11]. This causes difficulties in drying equipment design and processing and results in frequent downtime and high losses of product due to the adhesion of powder onto the equipment surfaces. Several research and review papers have been published in this regard. However, inadequate focus has been given to both the interfacial and interphase surface energies that contribute to stickiness.

TABLE 26.4
Dissociation Energy of Particle–Particle Cohesion

Interparticulate Forces	Dissociation Energy
Liquid bridges	Dependent on the composition of powder
Solid bridges	200–800 kJ/mol
Van der Waals forces	4–40 kJ/mol
Electrostatic forces	Dependent on the particle surface, shape, external electric field, and prehistory
Mechanical interlocking	Variable depending on shape, bulkiness, and flakiness

Source: Barbosa-Cánovas and Juliano [8].

TABLE 26.5
Surface Wetting Tension of Various Solid Materials

Surface	Wetting tension (mN/m)
Polytetrafluoroethylene (Teflon)	18
Polydimethyl silioxane (silicone)	21
Polyethylene	31
Polystyrene	33
Polyvinyl chloride	39
Cured epoxy resin	43
Polyethylene terephthalate (PET)	43
Nylon-6,6	46
Stainless steel	71
Aluminum	90
Soda glass	65

Source: Pocius [9].

The wetting of the solid surface is related to the surface energy of the adhesive and adherend (also called substrate). If the liquid wets the surface, it can spread out on the solid surface. If the adhesive has low energy levels or low surface tension, it is absorbed by high energy level solids; the contact angle decreases and the wetting is effective. If the surface of the solids has lower energy, the contact angle is high and the wetting is poor. In other words, to achieve wetting, the surface energy of the solid material should be higher than that of the liquid. The surface energy of various solid materials is listed in Table 26.5. Similarly, the surface energy of some liquids is presented in Table 26.6. Normally inorganic materials have higher surface energy than organic materials; this means that organic materials have poor wettability (fewer tendencies to cause sticking of materials). Metals have high surface energy; therefore materials tend to stick more on a metal surface. Polymers have low surface energy, therefore these are difficult to wet. One polymer, Teflon, has very low surface energy. Since water is a very high energy material (Table 26.6) and Teflon has a very low surface energy (Table 26.5), these events are energetically incompatible; therefore the wetting angle is very large. Currently, the drive in nanoparticle technology has been to attempt to manufacture an ultrahydrophobic surface

TABLE 26.6
Surface Energy of Some Liquids

Liquid	Surface Energy, (mN/m) at 25°C
Water	72
Epoxy resin	43
Glycerol	63
Ethylene glycol	47
n-hexane	18
Benzene	28.9
Nitrobenzene	43.9

Source: Pocius [9].

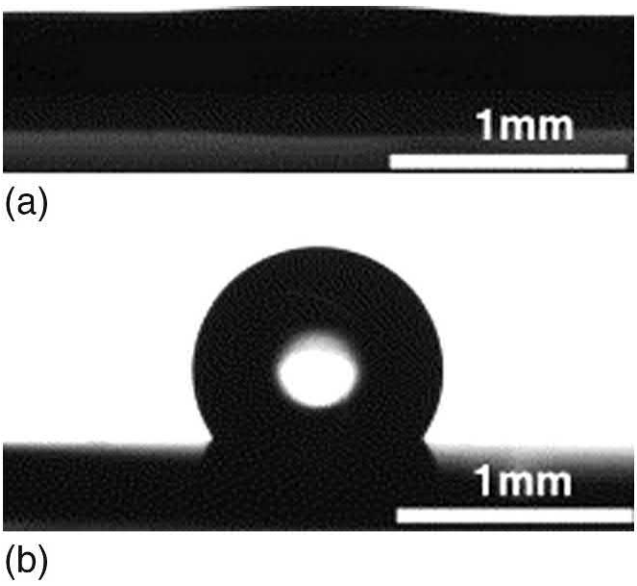

FIGURE 26.4 Nanoparticle coating results in low surface energy self-cleaning surface and water droplets on the surface (a) with nonfluorinated nanostructured TiO_2 coating, and (b) with nanostructured fluorinated TiO_2 coating, which gives a contact angle of the water drop around 150°. (From Burmeister et al. [13].)

with a very low energy level that can be nonadhesive and self-cleaned [12, 13]. An example is presented in Figure 26.4, which shows the effect of surface coating with nanostructured fluorinated and nonfluorinated titanium oxide (TiO_2). The coating with the former nanomaterial is rendered a contact angle of 150° [13]. A surface with a more than 150° water droplet contact angle has ultrahydrophobic, self-cleaning, and nonsticky properties. In the future, this material has potential applications in the food and powder processing (such as drying) industries handling sticky products.

26.3.3 Occurrence of Cohesion and Adhesion during Drying

Stickiness is encountered during drying and handling of some key dried food materials such as whey, lactose, protein hydrolysate, high-fat milk, fruit juices, honey, and high dextrose equivalent glucose syrups. Spray drying is the liquid food drying method where stickiness is the most common major issue. The powder may deposit on the wall or blockage may occur in the duct or cyclone. In spray drying the stickiness is not purely by adhesion onto the dryer wall. At the accumulation stage, the cohesive force between the particles can play a role, since the wall can be completely covered with the material. It is important to avoid the early stage of adhesion, which can act as a seed for further accumulation of underdried or sticky particles.

The concentration of solutes during drying can increase the surface tension, but the most influential factor is the rapid increase in viscosity. The increased viscosity results in increased time required to wet the wall because of the high resistance to flow. Thus the stickiness mechanism involves both the viscous flow of the droplets and surface energy of the droplets and dryer wall [14]. Once the product (as droplets) is in contact with the system, the liquid flow can be dictated by the surface tension or energy of the system. The liquid bridge between the dryer wall/product (adhesion) and product/product (cohesion) occurs as a function of time. If the contact area is very small and the drying continues, the dried particles may be carried away from the wall. To reduce the contact area, it is important that the wall material has a low surface energy. In many drying situations, stainless steel is used as the wall material because of its durability, ease of cleaning, and hygienic reasons, but this metal has a high surface energy (Table 26.6). Teflon (PTFE) has been found to be very useful in the handling and processing of food materials due to its extremely low surface energy (18 mN/m), relative inertness, high tensile strength, and thermal stability. There are many other polymeric materials that are hydrophobic and also have a low surface energy level. Due to their poor physical and thermal stability, and possible interaction with the food components, they are not used in many food processing situations, particularly in high temperature drying processes.

Many studies have sought to relate stickiness to the glass transition property of a drying food material. The glass transition normally signifies the conversion of an amorphous solid (or glass) to a rubbery state. In surface energy terms, a solid glass has low surface energy and is unable to stick on any other low energy solid surfaces. Due to the transition from glass to a rubbery (or liquid) state, the surface energy of the material increases and the molecules start interacting with the solid surface. In a food drying operation, the product is in a liquid or rubbery state and, due to the removal of plasticizer (water), the liquid/rubbery food is converted to the glassy state. If the food material does not go through the transition due to a higher drying temperature than the glass transition temperature, the product remains in a high energy sticky state. If this food comes in contact with a high energy solid surface, it can stick or cling to it [1].

The issue of adhesive/cohesive force is also important in the case of fluidized bed dryers handling pasty or solid particulates. The development of cohesive forces resulting from interparticulate liquid bridges can make the operation complex in fluidized and spouted beds [15]. The surface energy of dried or semidried particles can also be influenced by the electrostatic energy generated during their movement. Ciborowski and Wlodarski [16] reported the occurrence of electrical forces in fluidized beds. They found that electric charges that accumulate on solid particles may cause the adhesion of a layer of solid particles to the walls of the fluidizing equipment, the agglomeration of particles into larger aggregates, and the change of a fluidized bed into a channeling bed. Machowski and Balachandran [17] also stated that cohesive powders are generally much more difficult to transport due to short-range molecular forces (van der Waals) as well as electrostatic forces, which cause agglomeration and adhesion of particles, impeding the flow. The surface energies of particulates and binding agents are also very important factors in the agglomeration process applied to instant dry food powders.

26.3.4 Stickiness Testing Methods for Powders

26.3.4.1 Testing for Cohesion

26.3.4.1.1 Propeller-Driven Method

Originally developed by Lazar et al. [18], the propeller-driven method has been used by several researchers with or without modifications to evaluate the effects of temperature on powder stickiness (Figure 26.5). The tester basically comprises a test tube containing powder with known moisture content. The test tube is immersed in a water bath. A machine-driven impeller stirs the powder. When the temperature of the powder is slowly raised by increasing the water bath temperature, at the sticky point a maximum force of stirring is recorded. In a search for a simpler and more efficient technique, Özkan et al. [19] developed a viscometry technique based on the measurement of the torque required to turn a propeller inserted into powders.

26.3.4.1.2 Optical Probe Method

A method based on the changes in the optical properties of a free-flowing powder was reported by Lockemann [20]. The motion of the powder in a constantly rotating tube is observed with a fiber-optic sensor (Figure 26.6). The tube and sensor are all immersed in an oil bath to maintain the temperature. A sharp rise in reflectance of a freely flowing powder is observed at its sticky point.

26.3.4.1.3 Blow Test

Paterson et al. [21] attempted to develop a blow test for measuring the stickiness of powders (Figure 26.7). This method measures the velocity of air needed to blow a channel into a packed bed of powder, and the stickiness of powder is classified based on the air velocity range. The apparatus consists of a multisegmented circular distributor (sample holder) where the preconditioned sample is packed in the distributor.

26.3.4.1.4 Fluidization Method

Bloore [22] described a small fluidized bed set up to study the stickiness property of powder at different humidity and temperature conditions (Figure 26.8). The positive point of this method as compared to other tests is that the particulates are in a dynamic condition, which is closer to the spray drying and fluidized bed drying situations.

26.3.4.1.5 Cyclone Method

A cyclone technique (previously conceptualized and developed by Bhandari and Howes at the University of Queensland, Brisbane, Australia) is described by Boonyai et al. [23] and used to investigate stickiness behavior of food powders as a function of temperature and moisture conditions. It simulates the dynamic condition in a spray drying system (Figure 26.9). The powder particles are individually in contact with a preconditioned air stream and hence a rapid simultaneous heat and moisture transfer occurs at the surface. The cyclone consists of a detachable sample holder at the bottom. A few grams of sample are put in the sample holder for the test. Stickiness is observed within 1–2 minutes when particles become cohesive and stick to each other, and some adhere to the chamber wall due to adhesive force. If a longer time is allowed, all particles become completely immobilized. The testing time may also depend on the hygroscopicity of the material and particle size.

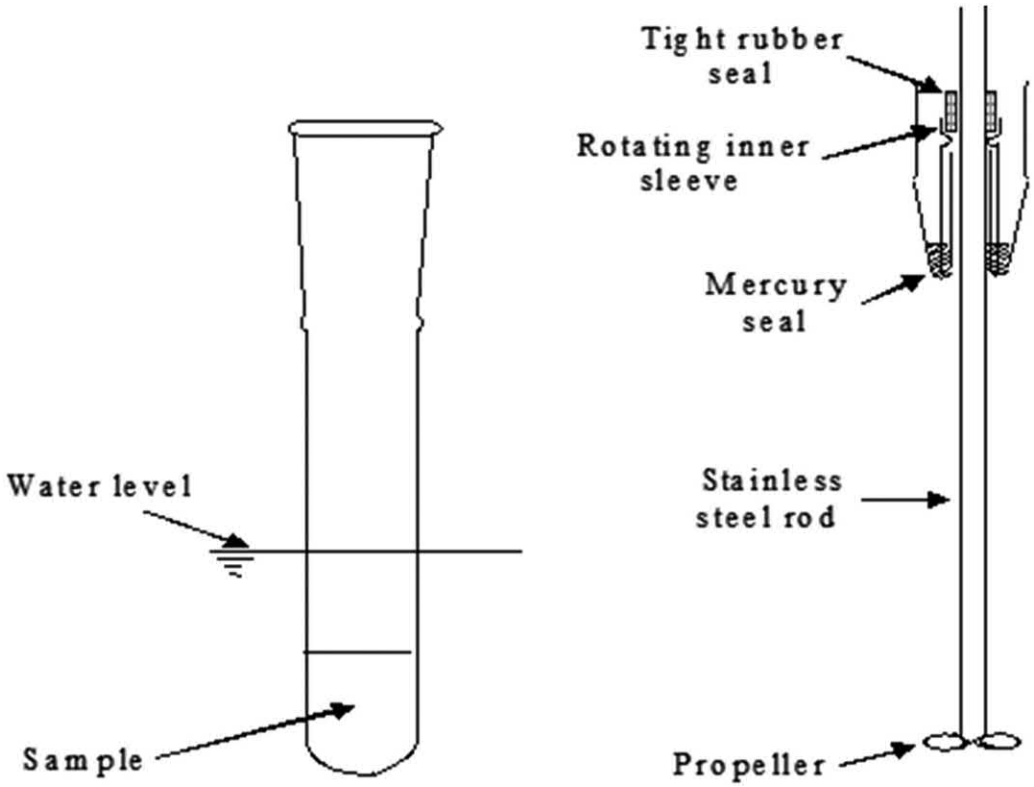

FIGURE 26.5 An early stickiness measuring device reported by Lazar et al. [18].

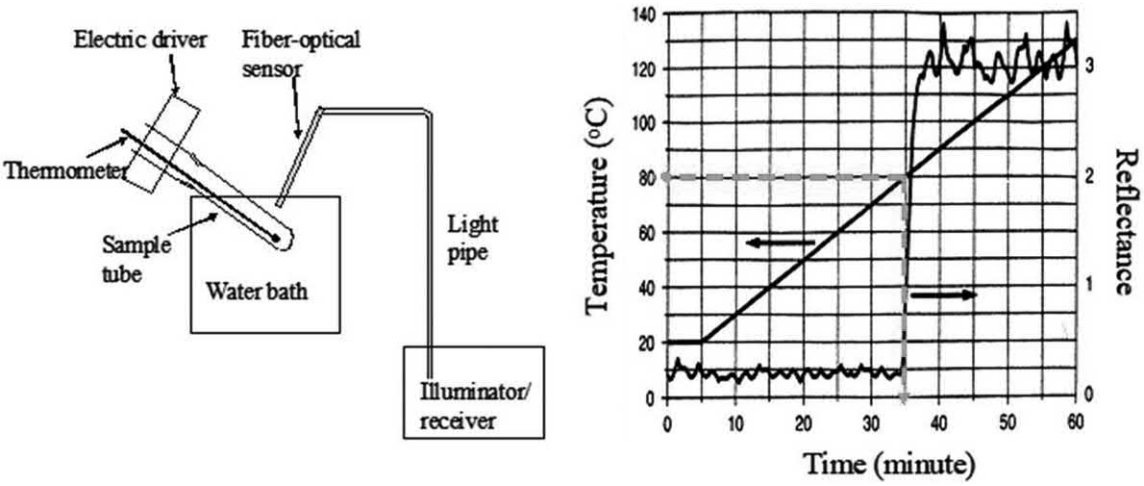

FIGURE 26.6 A stickiness device using an optical probe. (From Lockemann [20].)

26.3.4.1.6 Thermal Mechanical Compression Test (TMCT) Method

The thermal mechanical compression test assumes that the critical point of the stickiness of powders is at their rubbery state. During drying, the product is progressively transformed from liquid to rubbery and to glassy states. The product at the glassy state is totally solid in behavior and is not sticky. If the powder is not converted to the glassy solid state from the rubbery state during drying, it exhibits stickiness behavior (both adhesive and cohesive in nature). The reversible conversion from rubbery to glassy or vice versa is called glass transition temperature. The stickiness occurs when the powder glass transition temperature is below the outlet air temperature in the dryer. A common technique to measure the glass transition is differential scanning calorimetry (DSC). Since this technique is expensive and also requires expensive consumables (such as an aluminum pan), a simple technique called the thermal mechanical compression test (TMCT) was conceptualized and developed by Bhandari and Howes at the University of Queensland. The TMCT technique measures the phase change of a material based on mechanical changes during the transition (Figure 26.10a). The powder sample is axially compressed at constant pressure (1–3 kg/cm^2) until equilibrium, and the temperature is scanned in creep mode in a texture analyzer (such as TA.XT2). When the powder reaches its glass transition temperature, the probe displacement takes place and this point is considered as the stickiness point of that particular sample, as depicted in Figure 26.10b [24]. In dynamic and very short contact time situations, the stickiness usually occurs at 10–20°C above the measured glass transition temperature.

26.3.4.2 Testing for Adhesion

Surface properties of a droplet, such as film formation and dried wall material properties, can greatly influence stickiness. The film formation property once again is a function of drying conditions. Adhikari et al. [25] developed a rig for testing the *in situ* stickiness behavior of a drying droplet (Figure 26.11). There are two setups in this rig: one for measuring stickiness and the other for studying drying kinetics. Both of the setups are housed inside a glass chamber supplied with hot air with controlled flow, temperature, and humidity. The linear actuator with an appropriate step size is used to achieve the forward and backward

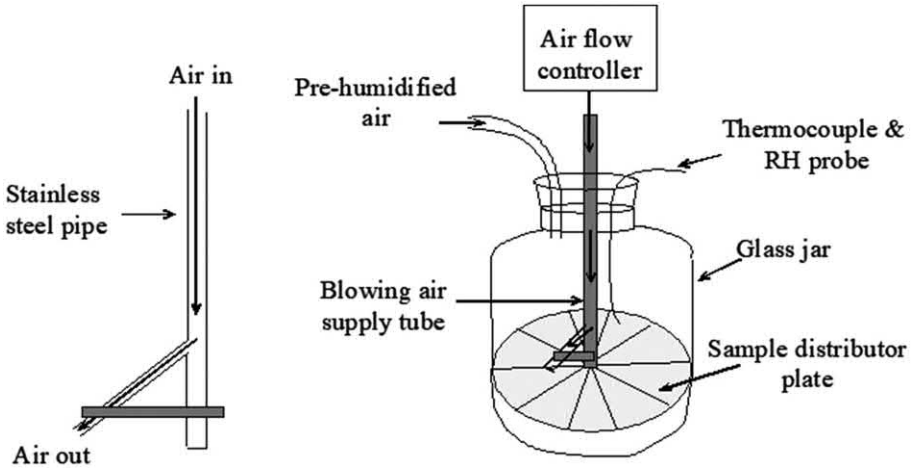

FIGURE 26.7 Blow test to measure the stickiness property of powders. (From Paterson et al. [21].)

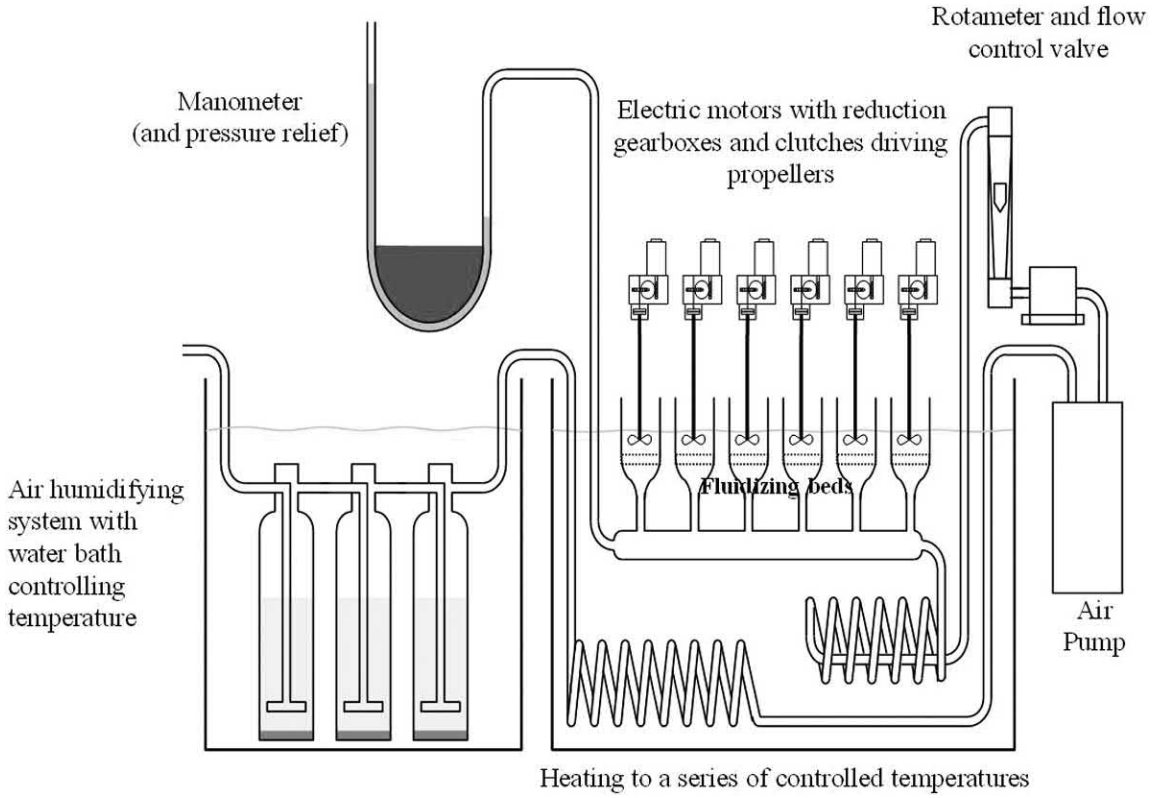

FIGURE 26.8 Fluidized bed stickiness testing device. (From Bloore [22].)

movements of the probe. The probe surface is made up of various materials (e.g., glass, stainless steel, Teflon, polyurethane). The tensile and compression force during stickiness testing and air temperature is continuously logged. The temperature history of the sample is recorded using micro-thermocouples. Images of bonding, debonding, and failure modes during the testing are captured using a color camera and recorded on a personal computer through a frame grabbing card. The same image capturing system is also used to monitor the drying droplet.

26.4 CAKING OF POWDERS

Stickiness is the preliminary stage of caking. In general, stickiness is an instantaneous phenomenon, whereas caking occurs over time. As previously mentioned, all amorphous products are metastable and therefore can potentially crystallize (for crystallizing species) during storage. The amorphous state is the most vulnerable to caking. The crystalline state is less prone to caking, however, the presence of an amorphous glassy layer around the crystal, dissolution of the outer surface of the crystal due to moisture absorption, and compaction can provoke caking of crystalline powder, too. Longer contact times increase the tendency toward sticking and caking, all other things being equal. Thus, a dried product with a relatively free-flowing property immediately after drying could also cake in a collection or packaging container over a period of time if the surface viscosity is still relatively low due to higher temperature or moisture levels. For this reason, the dried product needs to be cooled immediately to an appropriate temperature before packaging. Temperature changes and moisture migration in the bags during travel through different climate zones and consolidation pressure can cause undesirable caking in the powders [19]. The moisture fluctuations during day and night (or during shipment) also result in internal moisture migration in bulk solids due to the vapor pressure differentials in cold and hot conditions. This temperature fluctuation at the outer surface of packaging can be as high as 25°C in some climates. Due to the lack of conductivity of heat of the solids and cyclic heating and cooling, there is a formation of crust at the surface of the bulk powder. As stated earlier, the amorphous powders are most prone to caking due to their high hygroscopicity. Crystalline powder can also form cake due to surface dissolution and recrystallization. Mixed powders (coexisting amorphous and crystalline structure) have a mixed tendency to form cake. Various factors that influence the caking behavior of powder are depicted in Table 26.7.

26.4.1 Factors Responsible for Caking

There are four major causes of caking: (i) presence of liquid component, (ii) moisture absorption, (iii) crystallization, (iv) consolidation, and (v) deliquescence.

26.4.1.1 Presence of Liquid Component

Some of the food components can be in liquid state, such as fat in milk powders, oil in microencapsulated flavor powders, or viscous juice in dried fruits (such as raisins). In powder, such a liquid state should be in discontinuous phase, and so be encapsulated by the solid continuous

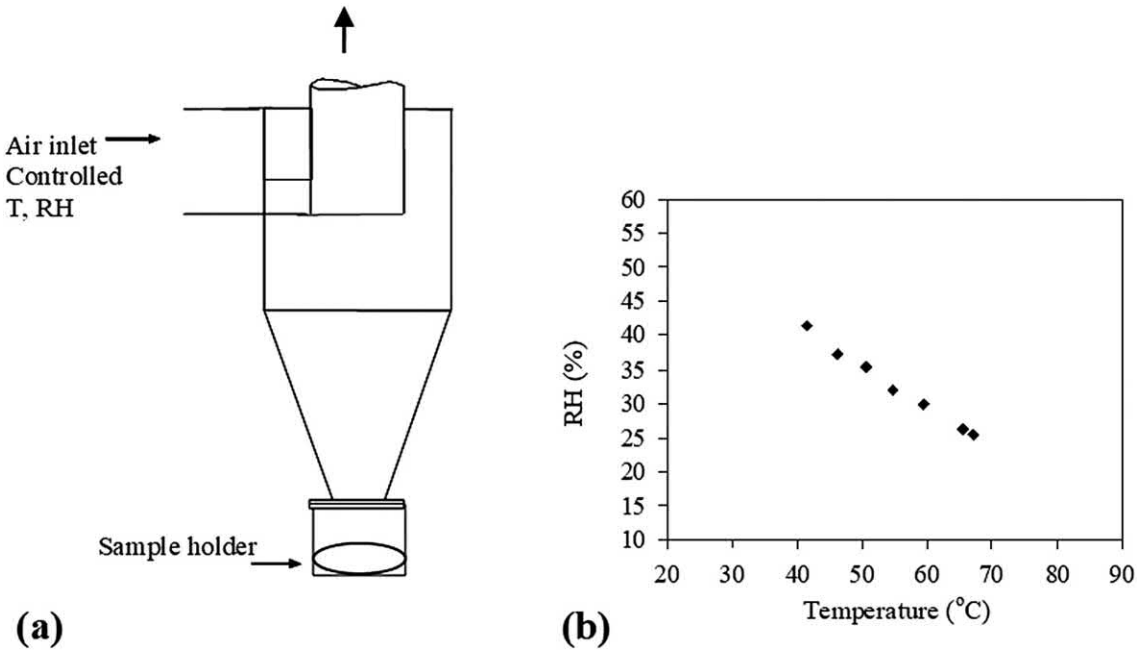

FIGURE 26.9 (a) Design of the cyclone chamber and sample holder. (b) Stickiness curve of skim milk powder as a function of temperature and relative humidity measured using a cyclone setup. (From Bhandari and Hartel [1].)

phase. However, leaching or breaking the structure can cause release and coalescence of such liquid at the surface of particles. This results in particles sticking together. This is manifested by decreased flowability and sluggish powder behavior. Free fat or oil in the powders cause caking, but the cakes are not as strongly held together as in the case of caking as a result of other factors, such as lactose crystallization in milk powder during storage. The increased temperature can also result in melting of the solid phase (fat to oil) or state change (glassy to rubbery), consequently resulting in caking of powder.

26.4.1.2 Moisture Absorption

Water absorption by the powder can cause the dissolution of the outer surface of the crystals or particles or the condensation of the capillary moisture in amorphous material (Figure 26.11). This eventually creates a liquid bridge. Upon dehydration, these liquid bridges are converted to strong solid bridges. The distance between the particles and hygroscopic property of the particle components influences the rate of caking. The higher the compactness of the particles and the finer the particles, the faster the cake formation.

26.4.1.3 Crystallization

Crystallization is also encouraged by water absorption by an amorphous powder (Figure 26.12). The extent of water absorption by powder depends on its sorption property. If a local portion of the product in a package picks up moisture, the glass transition temperature is locally depressed for that particular portion and the crystallization rate at that spot is accelerated. Formation of the lattice during crystallization generally excludes water molecules and the excess moisture is lost to the environment. Absorption of this ejected moisture at the surface of neighboring particles creates interparticulate liquid bridges resulting in caking [26]. Surrounding particles that absorb moisture are also crystallized and crystallization

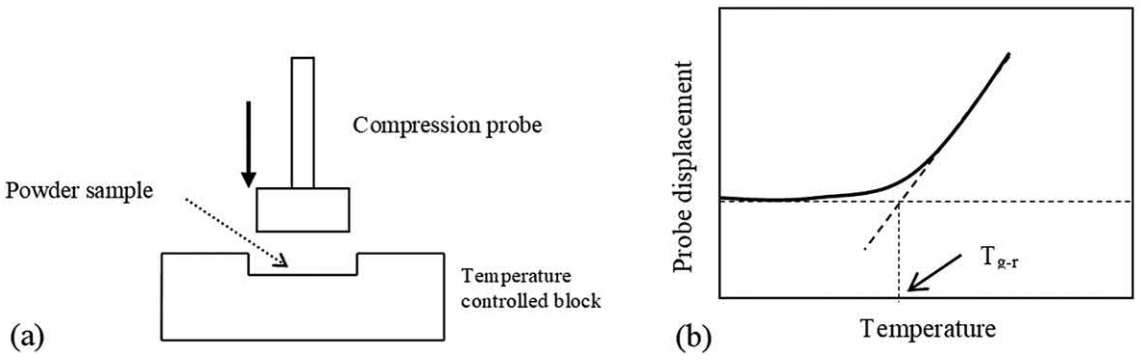

FIGURE 26.10 (a) Principles of measurement of stickiness of powder by the thermal mechanical compression test (TMCT) method. (b) Tg-r indicates glass rubber transition when the powder is scanned through temperature.

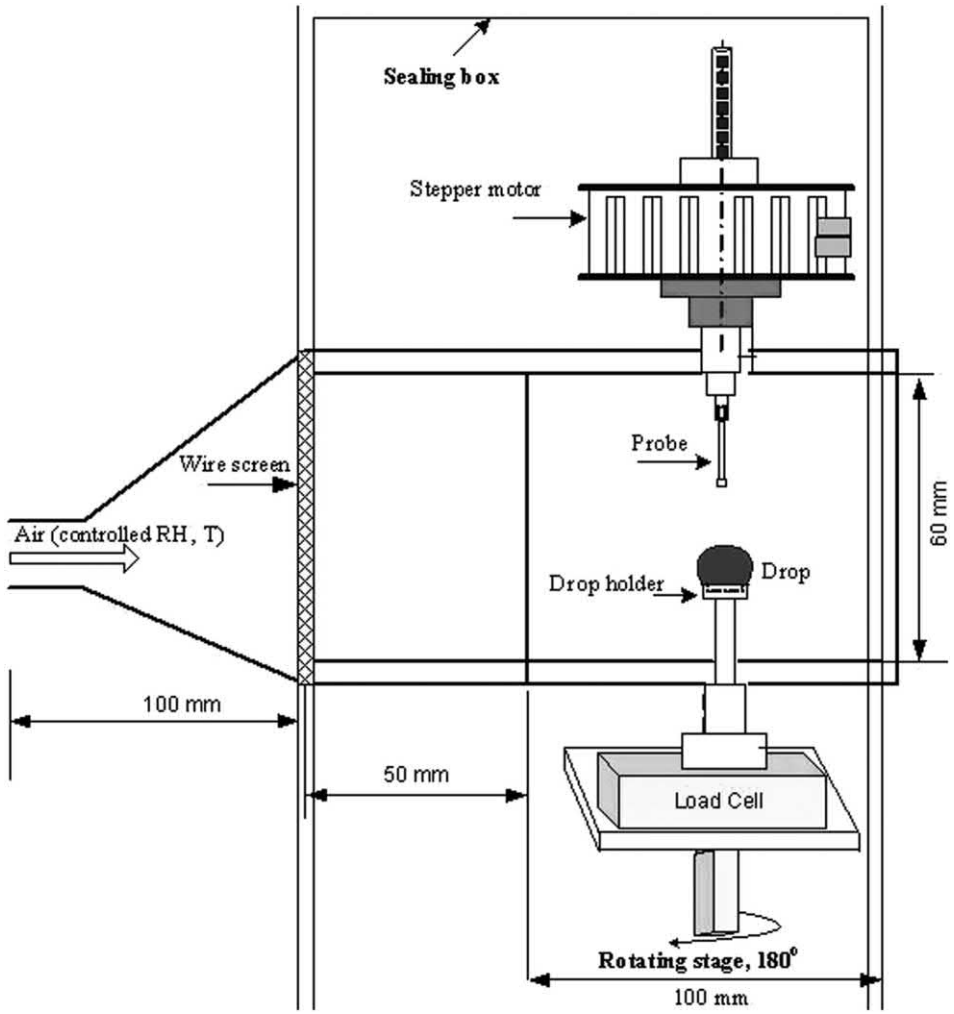

FIGURE 26.11 *In situ* stickiness testing device for a drying droplet.

TABLE 26.7
Process and Product Characteristics Influencing Caking Behavior of the Dried Foods (Powder)

Factors	Specific Details
Processing condition	Melting or dissolution of components in the powder due to high temperature processing or moisture addition
Microstructure	Extent of amorphous structure, very low average particle size (increased van der Waals attraction due to large surface area)
Product composition	Presence of liquid components at room temperature (such as oil, organic acids), presence of highly hygroscopic component, usually low molecular weight sugars, polyols, or organic acids
Storage condition	High humidity and/or temperature conditions causing melting and solidifying, glass–rubber transition, or crystallization of part or all of the components, powder consolidation due to overload, capillary condensation in crystalline particles

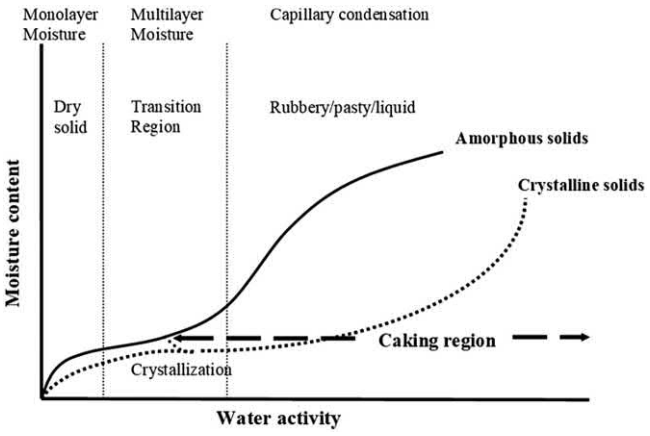

FIGURE 26.12 Indicative graph of water sorption isotherm of amorphous, crystalline, or crystallizing solids and their relationship to caking.

proceeds as a chain phenomenon. Crystallization of sugars is delayed by other ingredients present in the concentrated powder system by the same mechanism as in the supersaturated solution [27, 28]. The crystallization process rejects impurities including volatiles. Senoussi et al. [29] found a loss of diacetyl as a function of the rate of crystallization of lactose during storage. They found that when the lactose was stored at 20°C above glass transition, the amorphous product went through immediate crystallization and practically all diacetyl was lost after 6 days. Levi and Karel [30] also found an increased rate of loss of a volatile, 1-*n*-propanol, in an amorphous sucrose system as a result of crystallization.

26.4.1.4 Consolidation

Consolidation or compression of powders decreases the distance between the particles, and consequently van der Waals and other forces become predominant. This results in caking. This is more important if the powders are fine or brittle or break due to the compression force. The filling of the voids during compression, absorption of the moisture, leakage of liquid fraction, particle shape and size, and increased bulk density will contribute to caking during compression [31]. In some cases, this is desirable, such as during tableting where the binders are sometimes added in some powders to increase the tensile strength of the tablets. In the case of food, caking of the powder at the bottom of the stack is a commonly observed problem due to high consolidation force.

26.4.1.5 Deliquescence

Deliquescence is the liquefaction property of the crystalline materials (e.g., inorganic and organic salts, sugars, organic acids and bases, preservatives, flavor enhancers, or even vitamins) that occurs at a particular relative humidity, known as deliquescent relative humidity, which is specific for each type of deliquescence and temperature [32]. The deliquescent humidity is equal to the water activity (×100) of the saturated solution of that compound. Table 26.8 illustrates deliquescent relative humidity of several crystalline biological and inorganic materials at 25°C. At high relative humidity, water adsorption of solid materials results in the deliquescence of the solid surface and the formation of a concentrated thin film of solution on the surface of powder particles. The fusion of liquid films leads to the formation of liquid bridges between particles, which is the most common cause of the caking process of powders. Upon dehydration of powder particles (e.g., as exposing them to lower relative humidity), these liquid bridges are converted to solid bridges, as a result of recrystallization of solid materials, and consequently caking of powder is formed [33]. It is important to note that deliquescent relative humidity of a mixture of solid materials is always lower than that of any individual component. This causes the mixture of deliquescent solids to be more vulnerable to caking than individual components. It was reported that exposure of the mixture of citric acid anhydrous crystals and fructose crystals to 56% RH (25°C) for 16 h led to complete deliquescence of the fructose crystals and rounding of the edges of citric acid crystals, while at the same conditions citric acid and fructose crystals were both intact as they were separated [34]. Similar results were also reported for a mixture of amorphous maltodextrins and sodium chloride [35], a mixture of sodium chloride and sucrose [36], fructose–citric acid anhydrous and sucrose–sodium chloride systems [37], and a sucrose–citric acid mixture [38]. This is due to the capillary condensation effect at the contact point of the particles. The Ross equation was able to predict the deliquescent humidity of the binary mixture of solutes [34].

TABLE 26.8
Deliquescent Relative Humidity of Several Solid Materials (25°C)

Solid Materials	Deliquescent Relative Humidity (%)
Sucrose	85
α-Lactose monohydrate	95
β-Lactose	95
α-Glucose monohydrate	91
α-Glucose anhydrous	89-91
β-Glucose	74
Ascorbic acid	95
Malic acid	59
Sorbic acid	95
Sodium chloride	75
Potassium chloride	85
Monosodium glutamate	87
Magnesium chloride	33
Calcium chloride	28

Source: Mauer and Taylor [32].

26.4.2 Measurements of Degree of Caking

There are various methods employed to characterize the degree of caking by analyzing the change in the basic properties of the powders, such as flowability, angle of repose, interparticulate cohesion, size distribution, and particle morphology (Table 26.9). Among these techniques,

TABLE 26.9
Methods Employed to Measure the Degree of Caking

Method	Principle
Flowability	Discharge mass flow rate from a bin or funnel
Angle of repose	Heap angle along the horizontal plane (<40° for free-flowing powder)
Cohesion	Negligible shear stress for a free-flowing powder (Jenike shear cell method)
Caking index	Weight fraction retained by a mesh with an opening size of maximum particle size of the powder
Microscopic attributes	Ratio of interparticulate bridge diameter to particle diameter (microscopic techniques)

Source: Aguilera et al. [39].

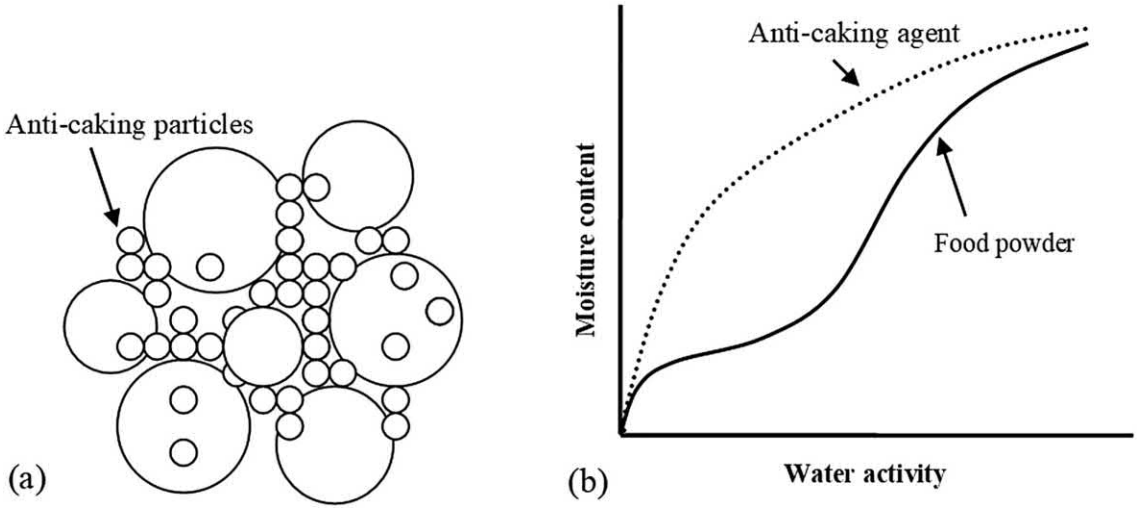

FIGURE 26.13 (a) Schematic representation of finer anticaking particles incorporated into larger particles. (b) Sorption isotherm property of anticaking agents.

microscopic observation or measurement of the increased size of the particles (indicated by the caking index) is the most appropriate method.

26.4.3 Anticaking Agents

Anticaking agents prevent the powder particles from sticking together. These agents are natural, inert, and bland in taste. Normally the anticaking agents are very fine powders (1–5 μm). Due to their smaller size, they tend to stick and coat bigger particles and separate two surfaces likely to adhere together (Figure 26.13a). A similar process is applied in dry coating of particles [40]. The van der Waals and electrostatic forces are responsible for sticking of fine particles to the bigger one. Anticaking powders have more surface area per unit weight; therefore, they absorb moisture faster than the actual powder. They have the capacity to absorb a large amount of water without exhibiting stickiness (Figure 26.13b). These can be inorganic and organic products. The inorganic powders include calcium and magnesium phosphates; aluminum, calcium, sodium, magnesium, potassium and ammonium salts of fatty acids; magnesium oxide; silicon dioxide (amorphous); calcium, aluminum, potassium and magnesium silicates or talc; bentonite (clay); and polydimethylsiloxane or dimethylpolysiloxane. These inorganic anticaking agents are mostly used in dairy powders and salts. The organic anticaking agents include microcrystalline cellulose, isomalt, fruit and vegetable fibers, corn starch, cereal (corn, rice) starches, and vegetable oil. These agents are widely used in fine icing sugars, shredded cheese products, dried fruit pieces, and fruit leathers. The level of addition of anticaking agents is normally less than 1% and should comply with good manufacturing practices (GMP). Criteria for GMP are (i) quantity shall be at the lowest level possible, (ii) it is not intended to accomplish any physical or technical effect in the food itself, and (iii) these need to be prepared and handled the same way as the food ingredient.

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27 Drying Methods Used in Food Preservation

Mohammad Shafiur Rahman and Conrad O. Perera

CONTENTS

27.1 INTRODUCTION

The preservation of foods by drying is the time-honored and most common method used by communities and the food-processing industry. The dehydration of food is one of the most important achievements in human history, making us less dependent upon a daily food supply even under adverse environmental conditions [1]. Drying in earlier times was done in the sun; now many types of sophisticated equipment and methods are used to dehydrate foods. During the past few decades, considerable efforts have been made to understand some of the chemical and biochemical changes that occur during dehydration and to develop methods for preventing undesirable quality losses. Foods can be divided into three broad groups based on the value added through processing by drying. In the case of cereals, legumes, and root crops, very little value is added per kilogram processed. More value per unit mass is added to foods such as vegetables, fruits, meat, and fish, and considerably more to high-value crops such as spices, herbs, medicinal plants, nuts, bioactive materials, and enzymes.

27.1.1 State of Water in Foods

The terms drying and dehydration are not synonymous. The US Department of Agriculture lists dehydrated foods as those with no more than 2.5% water (dry basis), while the term dried foods applies to any food product with more than 2.5% water (dry basis) [2]. The concept of bound and free water has been developed from drying principles, and it is important for dried products for their stability during processing and storage. A product containing no water is termed as bone-dry. Water in foods exists in different forms or states. Water in foods having properties different from those of pure water can be defined as bound water. In the literature different forms of bound water are defined [3], for example, unfreezable, immobile, monolayer, and non-solvent water. However, the fraction of bound water depends on its definition and the measurement techniques used to measure it [3]. The binding energy of different states of bound water affects the drying process since it requires more energy to remove bound water than free water.

27.1.2 Endpoint of Drying

Equilibrium in the drying system is the ultimate endpoint for the process. Water activity is commonly used to estimate the equilibrium point in the cases of thermal and osmotic drying processes. In mechanical dewatering, the magnitude of the applied force and rheological properties of the foods affect the equilibrium point. Generally, meat, fish, and dairy products are dehydrated to a moisture content of 3% or less, vegetable products usually to 5%, and cereal products frequently to as much as 12% [4]. A maximum moisture level is usually established for each dried product separately based on desired acceptable quality after drying and during storage. Different attributes of quality can be targeted; thus the endpoint should be determined based on all aspects, such as safety first and then consumer acceptance.

27.1.3 Heating Methods in Drying

Heating of air by electric heater or flue gas is the conventional heating method used for drying processes used in foods. In this case, heat transfer from the gas to the product occurs mainly through convection. The heating method is another important aspect of drying in terms of quality as well as energy cost. Microwave, infrared, radiofrequency, refractance window, and dielectric heating use the electromagnetic wavelength spectrum as a form of energy, which interacts with the materials, thus generating heat and increasing the drying rate dramatically. Dielectric drying uses frequencies in the range of 1–100 MHz, whereas microwave drying uses frequencies in the range of 300–300,000 MHz. Microwave heating is rapid, more uniform in the case of liquids, and more energy-efficient than the hot-air method [5]. Applying microwave energy under vacuum affords the advantages of vacuum drying and microwave drying, providing improved energy efficiency and product quality. The energy can be applied by pulsed or continuous modes. Pulsed microwave drying is more efficient than continuous drying. The use of electro-technology in drying is getting priority in the food industry to improve the drying efficiency as well as quality.

27.2 DRYING METHODS

Drying processes can be broadly classified, based on the water-removing method applied, as (i) thermal drying, (ii) osmotic dehydration, and (iii) mechanical dewatering. Thermal drying is one of the most widely used methods of drying foods. In this process, heat is mainly used to remove water from the foods. In thermal drying a gaseous or void medium is used to remove water from the material; thus thermal drying can be divided into three types: (i) air drying, (ii) low-air-environment drying, and (iii) modified-atmosphere drying. In osmotic dehydration, a solvent or solution is applied to remove water, whereas in mechanical dewatering physical force is used to remove water. Consideration of many factors should be given before selecting a drying process. These factors are (i) types of product to be dried, (ii) properties of the finished product desired, (iii) allowable temperature tolerance, (iv) the product's susceptibility to heat, (v) pretreatments required, (vi) capital and processing cost, and (vii) environmental factors. There is no one best technique of drying for all products [2, 6].

The mechanisms of moisture transfer depend mainly on the type or physicochemical state of food materials and the drying process. The food materials can be classified as (i) homogeneous gels, (ii) porous materials with interconnecting pores or capillaries, and (iii) materials having an outer skin that is the main barrier to moisture flow [7]. The type or structure of foods always played an important role in the drying process.

27.3 DRYING FUNDAMENTALS

In terms of transport phenomena, both heat and mass transfer processes are considered within the foods and outside atmosphere of drying. Hence, there are two resistances: heat transfer and mass transfer. During the *constant rate period*, it is assumed that there exists a thin film of water on the slice and there is no internal or external mass transfer resistance. Hence, the drying is controlled by external heat transfer. In the *falling rate period*, the drying is controlled by internal mass transfer resistance. The absence of a constant rate period indicates that the drying is controlled from the beginning by internal mass transfer resistance. The moisture content at which the drying period changes from a constant to a falling rate can be considered the critical moisture content. The critical moisture content depends on the characteristics of the foods and the drying conditions. The critical moisture contents varied from 0.78 to 0.83 (kg/kg, wet basis) for vegetables and 0.85 to 0.89 (kg/kg, wet basis) for fruits [8]. At high moisture contents, liquid flows due to dominated capillary forces. At decreasing moisture content, the amount of liquid in the pores also decreases and a gas phase is built up, causing a decrease in liquid permeability. Gradually the mass transfer is taken over by vapor diffusion in a porous structure. At the saturation point, there is no longer liquid available in the pores and mass transfer is taken over completely by vapor diffusion [9].

The moisture is transferred from the solid materials by diffusion or capillary mechanisms. In a diffusion mechanism, the driving force is the concentration gradient. Water diffusion can be in the form of liquid or vapor. In the case of liquid diffusion, osmotic pressure could be the driving force for water movement. In the capillary mechanism, the moisture moves due to surface tension force and does not conform to the laws of diffusion. A porous material contains a complicated network of interconnecting pores and channels, and at the surface mouths of pores of various sizes exist. As water is removed, a meniscus is formed across each pore, which sets up capillary forces by the interfacial tension between the water and the solid. Capillary forces act in a direction perpendicular to the surface of the solid. It has been suggested that a combined mechanism of capillary forces and vapor diffusion is responsible for moisture movement in the drying of potato [10, 11]. The drying experiments of Saravacos and Charm [8] with surface-active agents failed to show any importance of capillary forces during the dehydration of potatoes and other vegetables. Surfactants are known to reduce the surface tension of water, thus increasing the capillary forces in porous materials. Thus, capillary flow is not significant in the vegetables studied by Saravacos and Charm [8]. Waananen and Okos [12] showed that during drying of pasta at temperatures close to the boiling point, liquid flow dominates moisture transport at high moistures and vapor flow is significant only at low moistures. Achanta and Okos [13] reviewed the shrinkage of different biopolymers and concluded that shrinkage on drying is equal to the volume of moisture leaving; thus it is conceptually difficult to justify that capillary flow is important during the drying of high-moisture biopolymers.

The strength of capillary forces at a given point in a pore depends on the curvature of the meniscus, which is a function of the pore cross section. Small pores develop greater capillary forces than large ones; thus large pores tend to empty their water content first. In large pores, the capillary forces are small. The force of gravity is then large in comparison with the capillary forces, and there is a directional effect due to gravity [14].

27.3.1 Drying Curve

A drying curve usually plots drying rate (or moisture content) versus moisture content or drying time. There are three major stages of drying which could be observed in the drying curve (Figures 27.1 and 27.2):

1. Transient early stage, during which the product is heating up (transient period)
2. Constant or first period, in which moisture is comparatively easy to remove (constant rate period)
3. Falling or second period, in which moisture is bound or held within the solid matrix (falling rate period)

Typical drying rate curves are shown in Figures 27.1 and 27.2. The moisture content at which the change from the first to the second period occurs is known as the critical moisture content.

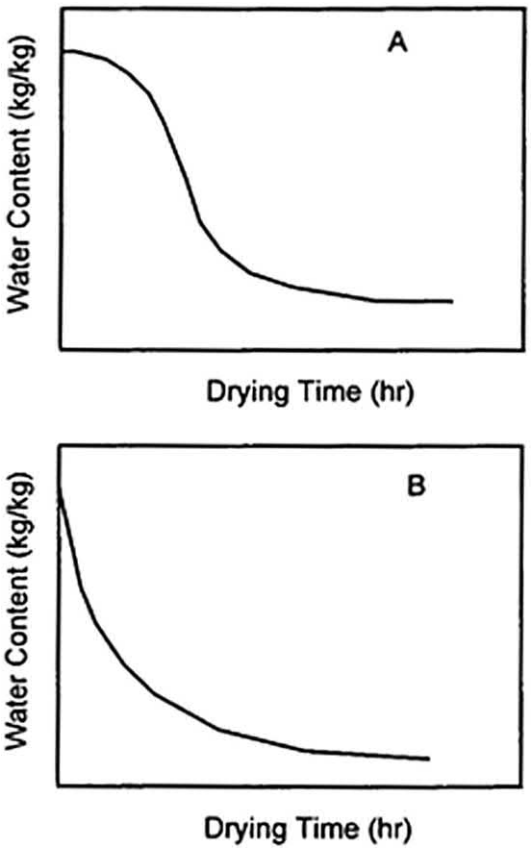

FIGURE 27.1 Typical drying curves (water content versus drying time): (A) with a lag period, (B) without a lag period.

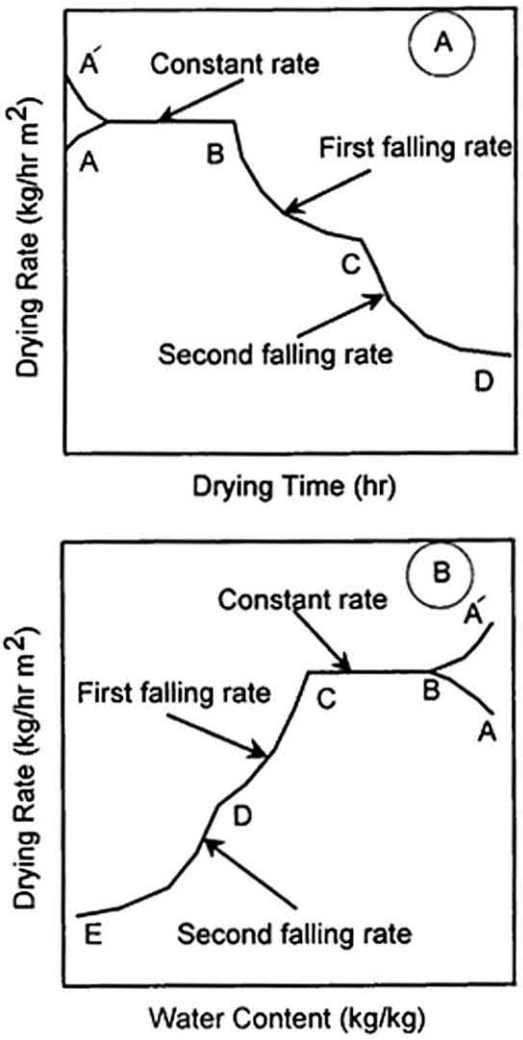

FIGURE 27.2 Typical drying rate curves: (A) drying rate versus drying time, (B) drying rate versus water content.

Typically, two falling rate periods are observed for both hygroscopic and non-hygroscopic solids [15]. The first falling rate period is postulated to depend on both internal and external mass transfer rates, while the second period, during which drying is much slower, is postulated to depend entirely on internal mass transfer resistance only. The slower rate may be due to the solid–water interaction or glass–rubber transition [13].

The drying behaviors of food materials depend on the porosity, homogeneity, and hygroscopic properties. The immediate entrance into the falling rate is characteristic of hygroscopic food materials. Lee et al. [16] studied the effect of sodium sulfate on the surface evaporation of a porous medium during the constant rate period. The drop in the drying rate was significant due to the decrease of surface vapor pressure and the change of liquid surface curvature due to meniscus effects by surface tension.

27.3.2 Energy Aspects of Air Drying

Drying is one of the most energy-intensive processes in the food industry. Apart from the rise of energy costs, legislation on pollution and sustainable and environmentally friendly technologies created greater demand for energy-efficient drying processes in the food industry. Thus, novel thinking in the technology of drying methods and dryer design is evident. The food industry could save a lot of money by avoiding costly energy waste. Improving energy efficiency by only 1% could result in as much as a 10% increase in profits [17]. Conducting an energy survey is the traditional way to approach the problem. The energy survey analyzes the energy defect level at each stage of processing and strategies for their remedy [17]. It is an inspection, survey, and analysis of energy flows in the system, which can enable the reduction of energy input without negatively affecting the output. It is a reliable, rational, and systematic approach to quantifying energy usage and energy efficiency [18]. The use of energy management, energy-saving technologies, and energy-saving policies are the keys to achieve savings [19, 20].

Microwave drying is an efficient method, and it provides high drying rates, high energy efficiency, and improved product quality [21–26]. It makes the core temperature of foods higher than the surface temperature, and this causes water transport to the surface and forms a porous structure [27, 28]. Air drying can be coupled with microwave drying. Microwave drying requires a high-temperature gradient between the core and surface, while air drying requires a high surface temperature to evaporate surface water. Optimization of the two different energy sources needs to be done in order to achieve the desired performance [29]. Material temperature, temperature difference, and size of the sample were optimized for desired quality of the dried products [30].

The use of infrared (IR) energy in combination with hot-air drying has attracted interest for the drying of foods due to the synergistic effect of infrared radiation on air drying [31]. It improves the final product quality, reduces the overall energy consumption and drying time, and reduces the total operating cost as compared to convective hot-air drying [32, 33]. However, appropriate combined strategies need to be followed to reduce energy consumption and to improve product quality [32].

27.3.3 Energy Losses in Air Drying

Heat losses during drying can be grouped as heat loss with the exhaust air, heat loss with the product, radiation heat loss from the dryer, heat loss due to leakage of air from the dryer, and heat loss due to over-drying of products. Table 27.1 shows the possible energy savings for walnut dehydration. Re-circulating exhaust air in grain dryers is popular because of its energy conservation and effect on grain quality. The higher-humidity air damages grains to a lesser extent than low-humidity air.

Grains are severely damaged by high drying temperatures. Thus, by changing the dryer design energy losses can be avoided while achieving higher product quality. Energy can be saved by (i) reducing drying time or increasing throughput (better control), (ii) avoiding heat losses with exhaust air and dried products, (iii) avoiding over-drying of products (i.e.

TABLE 27.1
Energy Savings for Walnut Dehydration

Method	Possible Savings (%)
Preventing over-drying	25–33
Recirculation of drying air	25
Reducing airflow rate	≤25
Improved burner design and operation	≤10
Insulation of drying	3–4

Source: Strumillo and Lopez-Cacicedo [35]

optimum drying with proper control), (iv) avoiding leakage of air through doors and seals, (v) increasing heat transfer efficiency by checking fouling, (vi) avoiding improper and damaged insulation around dryer, air ducts, heat exchanger, and burner, and (vii) heat recovery from exhaust gas and dried product [34]. The potential for energy conservation by design and changes in drying operation is significant. The energy could be recovered from exhaust air, heat exchangers (pipe and plate types), thermal wheel, heat pipe installation, and runaround coil. These methods recover mainly sensible heat from the exhaust, while most of the heat is lost with the latent heat of water vapor in the exhaust air [35]. This latent heat can be recovered by condensing out water using a refrigeration system. However, a refrigeration system will consume extra power before further use. Among these methods, the heat pump dryer (using a dehumidifier) has a high potential for use in the food industry (discussed later). In addition, improved options for dryer design can increase energy efficiency. These include the appropriate selection of dryer types and operating temperature, energy switching, hot-air recirculation, thermal insulation, and appropriate flow control inside the dryer [36, 37]. The broad classes of thermal drying are air-drying, low-air-environment drying, and modified-atmosphere drying.

27.4 AIR DRYING METHODS

In the case of air drying, atmospheric air is used as a drying medium, and heat in different modes could be applied in the process.

27.4.1 Sun Drying

In the past, only sun drying was used for drying. In this process, foods are exposed to the sun by placing directly on mats or stone or hanging in the air. The main disadvantages are (i) contamination from the environment, (ii) product losses and contamination by insects and birds during drying, (iii) the need for more floor space, (iii) difficulty in controlling the process, and (iv) production of bad smells for the neighbors. Where the climate is not particularly suitable for air-drying or when better quality is desired, mechanical air-drying is mainly used. However, sun drying is the cheapest method of drying foods. Nowadays, solar and mechanical air-drying is widely used commercially.

27.4.2 Solar Drying

Solar drying is a further extension of sundrying by using radiation energy from the sun. Solar drying is a non-polluting process and uses renewable energy. Moreover, it is an abundant energy source that cannot be monopolized [38]. Solar drying has several drawbacks, which limit its use in large-scale production. These are the need for large areas of space and for high labor inputs, the difficulty in controlling the rate of drying, and insect infestation and microbial contamination [6, 38]. More options in designing are now available in the literature in order to avoid or reduce the above difficulties [39]. Solar dryers can be classified into three major groups (Table 27.2): (i) direct, (ii) indirect, and (iii) mixed type.

27.4.2.1 Direct Solar Dryer

There are different types of direct solar dryer designs available. In a wooden box type solar dryer, the sides and bottom of the cabinet are painted black internally to absorb solar radiation, which is transmitted through an inclined glass cover. Ventilation holes are included in the bottom and upper sides of the walls. Food samples are spread on a mesh tray. The warm moist air passes through upper ventilation holes by natural convection, creating a partial vacuum and drawing fresh air through the base holes [39]. Other designs are presented by Jairaj et al. [39]. Chauhan et al. [40] reviewed the applications of software in designing solar drying systems.

27.4.2.2 Indirect Solar Dryer

In the case of indirect natural solar dryers, a solar air heater is coupled with the drying chamber and the air is circulated to the drying chamber by natural convention. In order to enhance the air circulation, different designs such as a chimney and top hood are used [39]. In the forced convection, air is heated first by a solar collector and then force-circulated to the drying chamber by a blower or fan [41]. The specific energy consumptions of ghost chili and ginger were observed

TABLE 27.2
Classification and Different Types of Solar Dryer

Direct Type	Indirect Type	Mixed Type
Cabinet	**Natural Convection Type**	Additional heater and fan
Staircase	Exit opening	Photo voltaic cabinet
Glass roof	Chimney	Photo voltaic greenhouse
Foldable	Chimney and top hood	
Greenhouse	**Forced Convection**	
	Air fan cabinet	
	Greenhouse	
	Geodesic dome	
	Tunnel	
	Obstacle solar collector	
	Multilayer rack	

Sources: Jairaj et al. [39], Mustayen et al. [134], Chauhan et al. [40], Kumar et al. [135]

as 18.7 and 8.8 kWh/kg, respectively [42]. Different design options are compiled in the literature [39].

Solar-assisted solid desiccant was used for the drying of crushed oil palm fronds [43]. In this system, solar energy was used to heat water with a solar collector and heat was transferred to the air through two heat exchangers: one used for regeneration of the desiccant wheel and another one to heat air after dehumidification. The drying time was reduced to 40 min instead of 30 h sun drying (moisture from 69 to 29%). A solar crop dryer was developed with phase change thermal energy storage [44, 45] and a packed-bed thermal energy storage system [46–48].

27.4.2.3 Mixed Type Solar Dryer

In the mixed type, an additional heater and fans are used. Solar photovoltaic cells are also used to generate electricity for the air circulation fan [39]. Sablani et al. [49] studied the performance of open rack, convection created by a fan operated by solar battery, and multi-rack dome dryer. In addition to the dryer performance, quality attributes of dried sardines were assessed by determining yeast, mold, and bacterial counts, peroxide value, and color. A significant variation in drying rates and quality attributes was observed. The dome drying could use a multi-rack tray in a big dome to increase the floor space for high loading, efficient use of energy, and better control of the process.

27.4.3 In-Store Drying

In-store drying can also be called low-temperature in-bin drying. It may be used where grain remains in store until milled or sold. Weather conditions in tropical climates are less favorable for in-store drying due to high ambient temperatures and relative humidity values. Two-stage drying can produce good quality by preventing discoloration of high-moisture grain and reducing cracking of dry kernels.

27.4.4 Convection Air Drying

Cabinet- and bed-type dryers (i.e. kiln, tray, truck tray, rotary flow conveyor, and tunnel) fall into the first generation [2]. This is the simplest drying technique, taking place in an enclosed and heated chamber. The drying medium, hot air, is allowed to pass over the product, which has been placed on open trays. Convection drying is often a continuous process and is most often used for products that are relatively low in value. Air-drying is usually accomplished by passing air at a regulated temperature and humidity over or through the food in a dryer. Factors that affect the rates of drying are temperature, humidity, air velocity and distribution pattern, air exchange, product geometry and characteristics, and thickness. The sample is usually placed on mesh trays in one layer or in bulk bed or hung from a string for better air circulation over the product. The air circulation can be horizontal or vertical to the layer or bed.

The structure and composition, such as the fat content, of a product affect the drying rate. In general, the hotter the air temperature, the faster is the drying rate, and similarly, the higher the velocity the higher the drying rate. The lower the air humidity, the higher the drying rate. The relative humidity (i.e. a measure of dryness) is lower when the air temperature is raised. The dryer must expel air to get rid of moisture, thereby allowing new, lower-humidity air to enter the system. However, this process causes heat loss from the dryer. In many cases, two or more stages with different conditions could be used, for example, initial drying at 90°C and then a second or final stage at 60°C. In the case of grains, re-circulating exhaust air in dryers is popular because of its energy conservation and beneficial effect on grain quality.

27.4.5 Explosive Puff Drying

Explosive puff drying uses a combination of high temperatures and high pressure and a sudden release of the pressure (explosion) to flush superheated water out of a product. This method gives the product good rehydrability. However, the high heat can degrade the food quality, and the explosion puffing may compromise product integrity [6]. An optimization of explosion puff drying with superheated steam on granny smith apple chips was reported by An et al. [50].

27.4.6 Spray Drying

Spray drying is used to remove water from free-flowing solution droplets, thus transforming them into a powder product. The fluid to be dried is first atomized by pumping it through either a nozzle or a rotary atomizer, thus forming small droplets with large surface areas. The droplets immediately come into contact with a hot drying gas, usually air. The liquid is very rapidly evaporated, thus minimizing contact time and heat damage. Disadvantages include that the size of the equipment required to achieve drying is very large, and very oily materials might require special preparation to remove excessive levels of fat before atomization [6]. Ultra-sonication in the chamber can be used instead of complex atomization to produce small-diameter droplets in spray drying.

27.4.7 Fluidized-Bed Drying

This technique involves the movement of particulate matter in an upward-flowing gas stream, usually hot air. Fluidization mobilizes the solid particulates, thus creating turbulences on the solid surfaces, which increases the drying rate. The hot gas is introduced into the bottom of a preloaded cylindrical bed and exits at the top. In some cases, a vibratory mechanism is used to increase the contact of the product with the hot gas. Fluidized-bed drying is usually carried out as a batch process, and it requires relatively small, uniform, and discrete particles that can be readily fluidized [6]. The main advantages of fluidized-bed drying are uniform temperature and high drying rates, and thus less thermal damage. A rotating chamber is also used with fluidized-bed drying, thus increasing the centrifugal force to increase the drying rate and mixing. The use

of a solid carrier, such as sea sand, and wheat bran could be used to prevent the biomaterial from deterioration due to thermal shock [51].

27.4.8 Spouted-Bed Drying

In a spouted-bed dryer, the heated gas enters the chamber at the center of a conical base as a jet. The particles are rapidly dispersed in the gas, and the drying occurs in an operation similar to flash drying. This works very well with larger pieces that cannot be dried in a fluidized-bed dryer [6].

27.4.9 Ball Drying

In this method, the material to be dried is added to the top of the drying chamber through a screw conveyor. The material within the drying chamber comes into direct contact with heated balls made from ceramic or another heat-conductive material. Drying occurs primarily by conduction. Hot drying air is passed through the bottom of the chamber. When the product arrives at the bottom of the chamber, it is separated from the balls and collected [6].

27.4.10 Rotary-Drum Drying

Rotary-drum dryers are cylindrical shells 1–5 m in diameter, 10–40 m in length, and rotating at 1–8 rpm with a circumferential speed of approximately 0.2–0.4 m/s. These conditions depend on the product types used for drying. They are designed to operate at a nearly horizontal position, inclined only by 2–6° to maintain the axial advance of solids, which are fed from the upper end of the dryer body [52].

27.4.11 Drum Drying

This technique removes water from a slurry, paste, or fluid that has been placed on the surface of a heated drum. The dryer may be comprised of either a single or a double drum. Drum drying is typically a continuous operation, and care must be taken to ensure that the product that is to be dried adheres well to the drying surface; in some cases, it may be necessary to modify the liquid product by additives to change its surface tension or viscosity [6].

27.4.12 Membrane Drying

Membranes are used to separate water vapor evaporated from the products, and the process is usually called membrane drying [53]. In the case of membrane drying, hydrophilic membranes (such as chitosan) are generally used because water is readily incorporated and diffused through these materials. The hydrophilicity is caused by functional groups present in the polymer chain [54]. This drying method could be carried out at room temperature and thus is beneficial to the product quality. In this method, foods are kept in one side of the membrane, and the permeation side of the cell is kept under vacuum; permeate vapor is collected in liquid nitrogen traps [53].

27.4.13 Electro-Hydrodynamic Drying

In the case of electro-hydrodynamic drying, air flowing is ionized between two electrodes applying a high voltage electric field, and it is known as corona wind or ionic wind [55]. This method passes charged air through a pointing electrode on the surface, and it enhances heat and mass transfer [56, 57]. This enhancement is due to the boundary layer disturbed by the ionic wind when it hits the surface of the wet food, causing reduced latent heat of vaporization [58]. In the case of quince slice, the selected quality attributes of electro-hydrodynamic drying were improved as compared to hot-air drying [59]. The average energy used in hot-air drying was 49 times as much as the average energy consumption of the electro-hydrodynamic method [60]. The drying rate of a wheat sample was significantly increased by increasing applied voltage and air velocity, and wheat protein conformation was significantly affected by hydrogen bonding pattern [61]. A combined electro-hydrodynamic and convection drying of mushroom slices showed that higher voltage or airflow velocity resulted in a higher drying rate, porosity and rehydration ratio, and a lower residual moisture content, while it caused a wrinkled and broken structure with higher shrinkage and shear strength [62]. Similarly, apple slices showed a higher drying rate with higher voltage and air velocity [63].

27.4.14 Intermittent Batch Drying

Progress is being made in intermittent drying, which can be applied to any direct dryer and batch dryer, such as tray dryers, convective dryers, conveyor dryers, fluidized-bed dryers, and spouted-bed dryers. Two strategies could be implemented: (i) time-varying or cyclic operating conditions, and (ii) step-wise change of operating conditions [64].

The main reasons for using the first strategy are (i) enhanced drying kinetics, (ii) reduced energy consumption, and (iii) improved quality [65–68]. In this strategy, internal moisture migrates to the material surface during the non-active phase of drying (i.e. tempering or non-active period). In the active period, heat input is applied to the drying medium. These two periods are carried out in an alternating mode. The drying rate is enhanced during the active period since surface water is increased during the tempering period. However, drying time is increased due to the passive period. This could be offset by lower energy consumption and better quality of the dried products [64].

In the second intermittent drying strategy, the stepwise change of operating conditions is applied to minimize energy requirements. The purpose is to gradually reduce the heat input since the drying process at the end is controlled by internal diffusion and external factors have limited effects [64]. However, the final temperature should not be as low as the equilibrium moisture content in the sample since it is temperature dependent [69]. In this stage, multiple modes of heat input, such as infrared, microwave, and radiofrequency, can be used to remove both surface and internal moisture simultaneously.

27.5 LOW AIR ENVIRONMENT DRYING

27.5.1 Vacuum Drying

The vacuum drying of food involves subjecting it to a low pressure and a heating source. The vacuum allows the water to vaporize at a lower temperature than at atmospheric conditions; thus foods can be dried without exposure to high temperatures. In addition, the low level of oxygen in the atmosphere diminishes oxidation reactions during drying. In general the color, texture, and flavor of vacuum-dried products are improved compared to air-dried. In some cases, the product quality is comparable to that of freeze-dried products. The potential of microwave-assisted vacuum and freeze-drying processes have also been explored for foods [70].

27.5.2 Freeze Drying

In freeze drying, the material that has been frozen is subject to a pressure below the triple point (at 0°C, pressure 610 Pa) and heated to cause sublimation of ice to water vapor. A schematic diagram of different states of water with triple point is shown in Figure 27.3. This method is usually used for high-quality dried products, which contain heat-sensitive components such as vitamins, antibiotics, and microbial culture. The virtual absence of air and low temperature prevent deterioration due to oxidation or chemical modification of the product. This method also gives very porous products, which results in a high rehydration rate. However, freeze drying is a slow and expensive process. The long processing time requires additional energy to run the compressor and refrigeration units, which makes the process very expensive for commercial use. Thus, it is mainly used for the high-value products [6]. Moisture in freeze-drying is usually removed by two mechanisms: (i) sublimation during the early stage until freezable water remained as ice in the sample, and (ii) vacuum drying when the sample contains unfreezable solvent or bound water (i.e. final stage of drying).

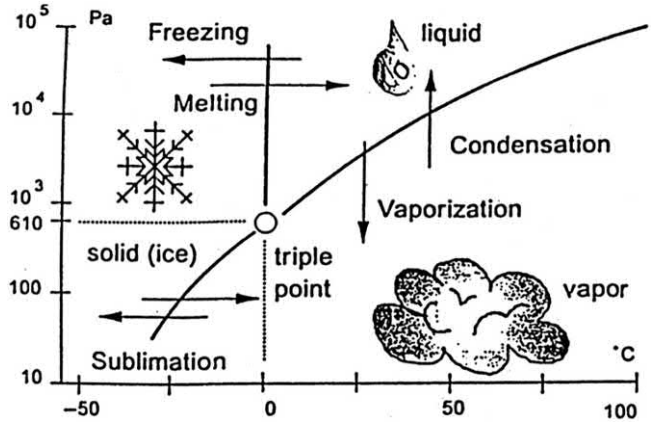

FIGURE 27.3 Schematic diagram of the different states of water showing triple point. (From Nijhuis et al. [133].)

27.5.3 Heat Pump Drying (HPD)

The heat pump dryer is a further extension of a conventional convection air-dryer with an in-built refrigeration system. The dry, heated air is supplied continuously to the product to pick up moisture. The humid air passes through the evaporator of the heat pump where it condenses, thus giving up its latent heat of vaporization to the refrigerant in the evaporator [71]. This heat is used to reheat the cool dry air passing over the hot condenser of the heat pump; thus the latent heat recovered in the process is released at the condenser of the refrigeration circuit and used to reheat the air within the dryer (Figure 27.4). The use of the heat pump dryer offers several advantages over conventional hot-air dryers for the drying of food products, including higher energy efficiency, better product quality, the ability to operate independently of outside ambient weather conditions, and zero environmental impact [71]. In addition, the condensate can be recovered and disposed of in an appropriate manner, and there is also the potential to recover valuable volatile components from condensate [72]. One of the main reasons for quality improvements in heat pump-dried products is due to the ability of heat pumps to operate at low temperatures. If a heat pump dryer is used at low temperatures (10–60°C) for highly perishable food products, adequate precautions need to be taken to prevent potential spoilage. There is also the potential to use a heat pump dryer as a modified atmosphere dryer to obtain better-quality products [72]. New developments in heat pump drying systems include the microwave-assisted HPD, modified atmosphere HPD, microwave-assisted modified atmosphere HPD, and desiccant-assisted HPD, all of which are discussed by Perera [71].

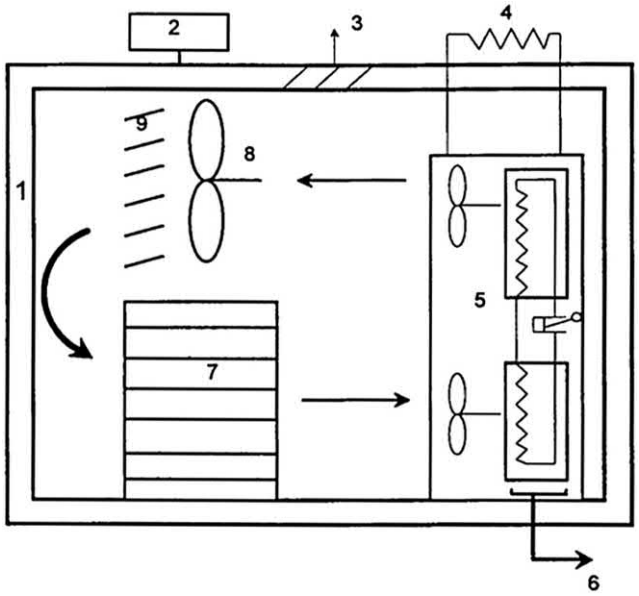

FIGURE 27.4 A schematic diagram of the operation of a typical heat pump dryer. 1, vapor-sealed and insulated structure; 2, humidifier; 3, overheat vent; 4, external condenser; 5, heat pump dehumidifier; 6, condensate; 7, product tray; 8, primary air circulation fan; 9, air distributor. (From Perera and Rahman [72].)

27.5.3.1 Energy Efficiency

The removal of water in its liquid state rather than the vapor state allows the latent heat of vaporization to be captured, and only a small amount of sensible heat is lost with condensate. The energy spent in drying is usually expressed as the "specific moisture-extraction rate" (SMER). The SMER for a well-designed heat pump dryer lies between 1 and 4 kg/kWh, whereas the SMER of a single-pass hot dryer is only 0.95 kg/kWh [72]. A general comparison of heat pumps with vacuum and hot-air drying is presented in Table 27.3.

27.5.3.2 Process Efficiency

Heat pump drying has the ability to operate at set conditions independently of outside ambient weather conditions. In addition, it is environmentally friendly, i.e. no gases or fumes are given off to the atmosphere. Since drying takes place in a closed system, a low air-leakage rate gives negligible heat loss.

Increasing the humidity in the drying air slows down the drying process but improves the energy efficiency [73]. In general, heat pump drying efficiency and capacity are dependent on temperature and humidity. In general, the SMER increases with an increase in humidity in the dryer [72]. In a conventional air dryer at low temperatures (10–30°C), it is not possible to run the drying operation due to high ambient relative humidity (0.70–0.90), but heat pump drying can be performed at these low temperatures since the relative humidity can be lowered to 0.10.

The thermal insulation and gas-tightness of the seals of the chamber structure are important in achieving high energy efficiency for the heat pump. In addition to the electrical energy required to drive the compressor, energy is also required to preheat the product and chamber structure, to drive the fan for primary airflow over the product that is to be dried, and to replace any heat loss through conduction and air leakages. The motors driving the fan and the compressor can be located within the chamber so that the residual heat produced by them is absorbed within the drying chamber instead of being lost to the atmosphere [72].

27.5.3.3 Progress and Applications

There are a number of technological problems to be overcome before the process can be applied in the food industry.

Capital cost: the capital cost of a heat pump dryer is higher than for a conventional hot-air dryer due to the additional refrigeration system requirement. However, its cost should be much less than that of vacuum or freeze drying.

Limited drying temperature: while low-temperature drying is a potential advantage, too low a temperature will limit the drying rate, which has implications for throughput. Also, slower drying rates at low temperatures may give rise to potential microbial growth problems [72].

Process control and design: like vacuum and freeze dryers, heat pump dryers are more amenable to batch drying because the drying takes place in a hermetically sealed container. The construction of continuous drying may involve high engineering modeling and design costs. Therefore, benefits need to be evaluated on the basis of cost rather than energy efficiency alone [72].

Innovative heat pump drying systems, such as multistage compression, cascade, tunnel, vapor adsorption, vacuum condition, and multiple evaporators in series and parallel, are presented [74–76]. In addition, heat pumps with two or more drying chambers in cylindrical mode can be used to dry different products in different chambers [77]. Heat pump drying can be incorporated with other drying methods, such as spray drying [78], and atmospheric frozen drying [78, 79], and solar drying [80]. It is possible to operate at various strategies, such as cyclic temperature, step-down temperature, cyclic pressure, and variable gas flow [81].

27.5.4 Superheated Steam Drying

Superheated steam is used as a drying medium. The main advantages of this type of drying are that it can provide an oxygen-free medium for drying, and process steam available in the industry can be used without any capital cost. An oxygen-free medium has the potential to result in high-quality food products, however, it is important to generate more information regarding quality improvement and processing efficiency. An et al. [50] optimized the conditions for instant pressure drop puffing with superheated vapor (IPDPSV) to the physical properties of granny smith apple chips and found that at 23% moisture content of pre-dried samples, puff drying at 122°C for 41 s gave the best color, porous structure, and suitable hardness compared to those processed by hot-air drying or freeze drying. Superheated steam provides other advantages, such as pasteurization, sterilization, and deodorization of dried products, which could give a high standard of hygiene, decontamination of hazards, provide higher drying

TABLE 27.3
General Comparison of Heat Pump Dryer with Vacuum and Hot Air Drying

Parameter	Hot Air Drying	Vacuum Drying	Heat Pump Drying
SMER (kg water/kWh)	0.12–1.28	0.72–1.2	1.0–4.0
Drying efficiency (%)	35–40	≤70	95
Operating temperature range (°C)	40–90	30–60	10–65
Operating % RH range	Variable	Low	10–65
Capital cost	Low	High	Moderate
Running cost	High	Very high	Low

rates in constant and falling rate periods, and the possibility of capturing desirable organic compounds from the condensate of effluent steam [64]. If products are not stable at 100°C, lower operation pressure could reduce the operating temperature. However, this could reduce the drying rate significantly [64].

27.5.5 Impingement Drying

Impingement drying is an old technology that has only recently been applied to food products. An impingement dryer consists of a single gas jet (air or superheated steam) or an array of such jets, impinging normally on a surface. There is a great variety of nozzles that can be used, and the selection of the nozzle geometry and multi-nozzle configuration has important relevance in the initial and operating costs and product quality [82]. Some characteristics of impingement drying include rapid drying, popularity in convection drying, and the large variety of nozzles available (multi-zones). Typically, the temperature and jet velocity in impingement drying may range from 100 to 350°C and 10 to 100 m/s, respectively [83].

27.5.6 Modified Atmosphere Drying

This is a new concept for drying foods for better quality and preserving their constituents using a modified atmosphere by flashing different gases, such as nitrogen and carbon dioxide [84]. Modified atmosphere heat pump dehumidifier (MA-HP) drying is a relatively new development described by Perera [85, 86] and Hawlader et al. [87–89]. The fact that heat pump dehumidifier (HP) drying is conducted in an enclosed, insulated chamber is made use of in the development of the MA-HP drying system. The air in the dehumidifier chamber is replaced with an inert atmosphere such as nitrogen, carbon dioxide, or their mixture. Replacement of the air inside the chamber is easily carried out, by exhausting the chamber using a vacuum pump and then breaking the vacuum using an inert gas. Therefore, vacuum exhaustion is a more cost-effective way to replace the air than by direct purging with the specific inert gas. Replacement of air with carbon dioxide or nitrogen by purging requires over 50 volumes to achieve an oxygen level of less than 0.5%. A schematic diagram of the MA-HP drying system is shown in Figure 27.5. This consists of a sealed drying vessel connected to the heat exchanger unit. The drying vessel has provisions for the introduction of nitrogen or for evacuation through a valve connection.

The MA-HP system has shown provision for introducing microwave energy for heating the product, through a slotted wave-guide running down the wall of the chamber parallel to the axis of the chamber. A PLC control panel connected to a remote PC and monitor controls the whole system. The product is carried on microwave-transparent plastic-trays stacked vertically on a rotating platform, which is mounted on a load cell, so that weight loss can be monitored and recorded on the PC. The current prototype is essentially a batch process, but it can be semi-automated depending on the products and pre-treatments required for specific products. Some of the pre-treatments may include vacuum infusion or osmotic dehydration before MA-HP drying. After the product is loaded on to the trays and stacked on to the platform, the

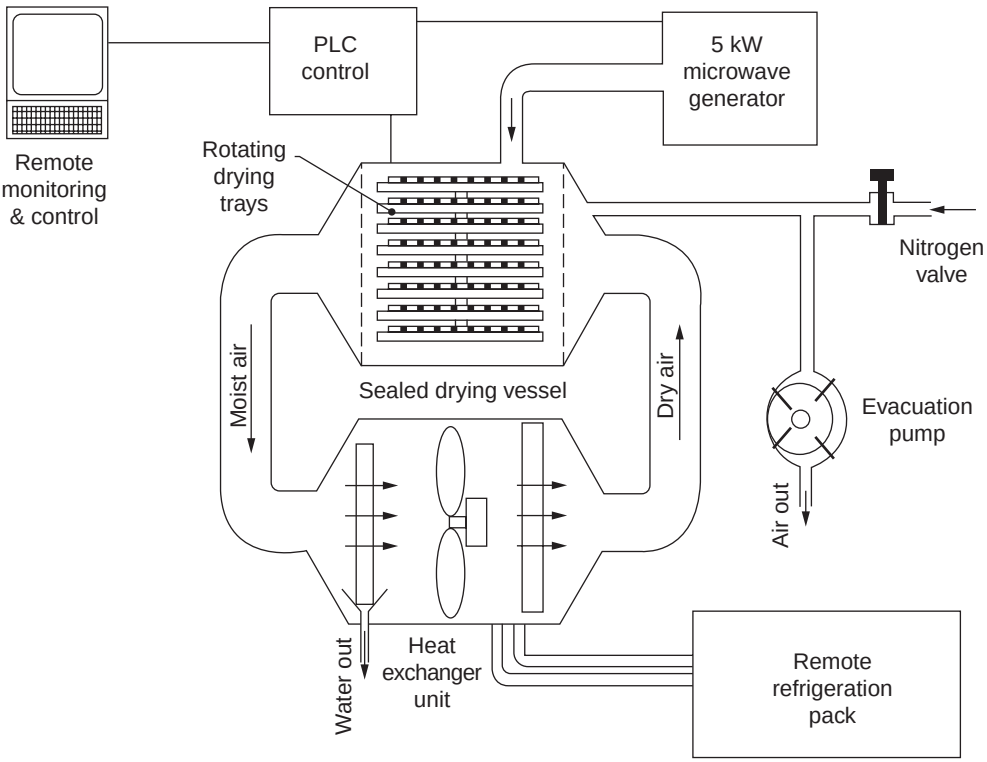

FIGURE 27.5 Schematic diagram of the MAHPD drying system. (From Perera [85, 86].)

drying chamber is evacuated to 600–700 Pa for 30 minutes, after which the vacuum is broken with the selected modified atmosphere and normal HP drying is carried out in this modified atmosphere. The use of microwave energy improves the drying rate. The surface temperature of the product is monitored using an infrared detector. Controls can be set for the microwave energy to cut off at a pre-set surface temperature of the product, so that overheating can be minimized.

Busic et al. [90] tested carbon-dioxide drying of basil for retention of bioactive components and improvement of taste and appearance. The moisture was reduced to 8.1 from 90.5 g/100 g sample within 3 days of drying when 100 bar pressure of carbon dioxide was used, while air-drying reduced moisture content to 23.0 g/100 g sample when dried for 26 days. However, drying at 80 and 100 bar did not show any significant effect on MA drying. The atmosphere in the MA drying can be modified by adding volatile compounds, such as ethanol, to reduce volatilization of volatile compounds in the foods inside the dryer [91].

It was observed that added ethanol 0.5% (v/v) in the air stream in the MA dryer could allow the retention of important volatile compounds of fresh pineapple aroma [91], and L-ascorbic acid in dried pineapple [92]. In addition to quality improvement, ethanol in the drying atmosphere promoted intense water evaporation, and thus reduced the drying time. The energy consumption of fluidized-bed drying of wheat kernels with carbon dioxide was reduced 20% and had a shorter drying time as compared to air fluidized-bed drying. This reduced energy consumption to 3% of input heat [93]. An additional energy savings of 4% of the heat load can be achieved for drying in carbon dioxide atmosphere at temperatures below 100°C due to the lower wet bulb temperature of carbon dioxide [64].

27.6 DRYING PRETREATMENTS

27.6.1 Blanching

Pretreatments are common in most of the drying process in order to improve product quality, storage stability, and/or process efficiency. In recent years an improvement in quality retention of dried products by alteration of the processing strategy and/or pretreatment has gained much attention. Blanching is a process of preheating the product by immersing in water or steam. The main purpose of blanching is to inactivate the naturally occurring enzymes present in foods since enzymes are responsible for off-flavor development, discoloration or browning, deterioration of nutritional quality, and textural changes in food materials. Blanching provides other advantages: (i) removing microorganisms from vegetable surfaces and intercellular spaces, thus reduces the initial microorganism load, (ii) cleaning raw food materials and facilitating preliminary operations, such as peeling and dicing, and (iii) improving color, texture, and flavor under optimum conditions [94–96]. Blanching may have disadvantages: it may cause changes to the texture, color, and flavor because of the heating process [97, 98], it increases the loss of soluble solids, such as vitamins [99, 100], especially in the case of water blanching, it may change the chemical and physical state of nutrients and vitamins [94, 101, 102], and it has adverse environmental impacts, such as large water and energy use and problems of effluent disposal.

Time and temperature of blanching are the important factors for achieving optimum quality of the dried product [103]. The normal blanching temperature varied from 80 to 100°C. Recently low-temperature and long-time blanching has been proposed for better texture and the retention of some nutrition components [104, 105]. The low temperature used is between 50 and 70°C. Blanching time correlated with flavor and sensory attributes of dried fruits and vegetables [98]. Limiting blanching time, rapid cooling, and alternative blanching methods and/or combinations may result in dried products with better flavor. Processing methods that include steam and/or microwave blanching and non-thermal enzyme inactivation such as high-pressure and ohmic heating may have potential for future blanching processes with fewer detrimental effects on flavor and maintenance of optimum texture.

27.6.2 Sulfur Dioxide Treatment

Sulfur dioxide preserves the texture, flavor, vitamin content, and color that make food attractive to the consumer. Sulfur dioxide treatment is used widely in the food industry to reduce the fruit darkening rate during drying and storage, and it preserves ascorbic acid and carotene. The sulfur dioxide taken up by the foods displaces air from the tissue in plant materials, softens cell walls so that drying occurs more easily, destroys enzymes that cause darkening of cut surfaces, shows fungicidal and insecticidal properties, and enhances the bright attractive color of dried fruits [106]. Permitted levels of sulfur dioxide and other additives (solutes) in dried foods vary from country to country. According to an IFT (1990) [107] expert panel, fruits can contain the highest levels of sulfur dioxide of all food products. The allowed limit is 2000 mg SO_2/kg of dried fruit.

Sulfiting treatment can be done by burning sulfur or soaking in a sulfite solution. The smoke of sulfur dioxide can be produced by burning sulfur with oxygen in the air and then circulating to the smoking chamber. Potential advantages in using a bisulfite solution are (i) decreased air pollution, (ii) better control of the sulfuring process, (iii) greatly shortened sulfuring time, and (iv) decreased desorption losses during drying [106].

The chemical reactions of sulfur dioxide when it is added to fruits and other food products are complex [108]. Sulfite can be bound or free in the food matrix. This bound sulfite is considered to have no retarding effect on product deterioration; thus it is important to know the factors that influence binding [108]. The amount of bound sulfite depends on pH, carbonyl groups of aldehydes, acetaldehyde, pyruvic acid, availability of oxygen, sugars, and starch [108–110]. Sulfiting and blanching are also used together as pretreatment [111]. Sulfiting ruptured and collapsed cells resulting in a smaller cell volume and hardness of dried samples [112, 113]. A number of factors

affect sulfur dioxide uptake by fruits and vegetables, including concentration and temperature of dipping solution, time of dipping, geometry and conditions of sample (i.e. peeled or unpeeled, whole or sliced), and agitation of solution [106, 114].

27.6.3 Salting or Curing

Salting or curing is a type of osmotic dehydration. Curing was originally developed to preserve certain foods by the addition of sodium chloride. In the food industry,y the application of curing is related only to certain meat, fish, and cheese products. Today sodium chloride, sodium, and potassium nitrite (or nitrate) are considered curing salts. Salting is one of the most common pretreatments used for fish products. Salting converts fresh fish into shelf-stable products by reducing the moisture content and acting as a preservative. In combination with drying, these processes contribute to the development of characteristic sensory qualities in the products, which influence their utilization as food [115]. Although curing was originally a mechanism for preservation by salting, over several millennia additional processes concomitant with curing have evolved, notably fermentation, smoking, drying, and heating. Curing may have different connotations: in meat, salt and nitrite are always added; in fish salt is always added, but nitrite only rarely; and in cheese, which always contains salt, but infrequently contains nitrate, and the term curing is applied to the production of desirable proteolytic and lipolytic changes. In the past half-century, cured products have been developed that are not stable unless refrigerated. Indeed, most cured meat products must be refrigerated to remain safe and wholesome, and during the past two decades even the packaging of many classes of cured products has become important in extending the period during which the product remains wholesome [116]. Cured meats can be divided broadly into three groups: unheated, mildly heated (pasteurized to center temperature of 65–75°C), and severely heated (shelf-stable after heating to 100–120°C) [116].

In addition to the curing salts and related processes mentioned above, additives collectively known as adjuncts are used in many cured meat products. These include ascorbates, phosphates, glucono-D-lactone, and sugars. Adjuncts are used primarily to obtain or maintain desirable changes, the ascorbates in connection with color and the others in connection with pH, texture, and in some cases flavor. Adjuncts may also affect safety. The concentration of each curing agent depends on the nature of the food products and on the technology used in individual countries [116].

27.6.4 Other Dipping Pretreatments

Dipping treatment with chemicals is also used in addition to blanching or sulfite treatment (Table 27.4). The dipping treatment is a process of immersion of foods in a solution containing additives. The concentration level is usually below 5%, and dipping time is usually below 5 minutes, whereas osmotic dehydration is usually carried out at higher concentrations and for long processing times. The main purpose of the dipping treatment is to improve drying characteristics and quality. Certain chemicals are used to enhance the rate of dehydration [97]. Among these compounds, methyl and ethyl oleate, or olive oil are the most common [117–120]. Methyl oleate has realized the greatest usage because of economics and its higher taste threshold. A carbonate–oleate combination was superior to their use alone in accelerating the drying rate [121–123]. A synergistic effect results from the combined use of alkali carbonates and methyl oleate for drying. When excess carbonate was used, drying was further accelerated. Sodium carbonate was less effective than potassium carbonate; however, the cost of sodium salt is about a fifth that of potassium.

TABLE 27.4
Chemicals Used for Dipping Treatment

Type	Compounds
Chemicals	
Esters	Methyl oleate, ethyl oleate, butyl oleate
Salts	Potassium carbonate, sodium carbonate, sodium chloride, potassium sorbate, sodium polymetaphosphate
Organic acids	Oleic acid, steric acid, caprillic acid, tartaric acid, oleanolic acid
Oils	Olive oil
Alkali	Sodium hydroxide
Wetting agents	Pectin, tween, nacconol
Others	Sugar, liquid pectin
Surfactants	
Nonionic	Monoglycerides, diglycerides, alkylated aryl polyester alcohol, polyoxyethylene sorbitan monostearate, sorbitan mono stearate, D-sorbitol, polyoxyethylene
Anionic	Sodium oleate, steric acid, sorbitan heptadecanyl sulfate, Dimethyl-benzyl-octyl ammonium chloride

Esters affect the waxy surface of fruits by altering the physical arrangement of the surface wax platelets, thus allowing moisture to more readily evaporate from the fruit. This was confirmed using electron microscopy for grapes and sweet cherries [124]. The hydrophilic groups on the wax surface increased by a reversible attachment of long-chain fatty acids and their esters. The increase in hydrophilic groups on the normally lipophilic wax surface would form a sequence of attachment sites to facilitate the transfer of water through the crystalline wax layer [125, 126]. The addition of potassium carbonate is necessary, possibly acting by saponification of fatty acids such as oleic, steric, and oleanolic acids, which are known constituents of grape wax. Table 27.3 shows the chemicals used for dipping treatment.

27.6.5 Freezing Pretreatment

Freezing treatment affects the drying process. The re-hydration rate of air- and vacuum-dried fruits and vegetables with freezing treatment increased to a level comparable to that for freeze-dried products [127]. It was also noticed that the longer the duration of freezing, the better the rehydration kinetics

of dried products. This was due to the formation of large ice crystals by slow freezing. In the case of blueberries, the temperature significantly affected the drying rate, whereas insignificant effects of cultivar and grade were observed [128].

27.6.6 Cooking

Cooking at different pressure levels before drying can destroy microorganisms and affect the physicochemical properties of dried products. The bacterial load on the final product can thus be much reduced, and cooked product can be minced and spread evenly on drying trays with much less trouble than raw material. Pre-cooking is usually used for rice, beef [129], fish, and beans [130]. The formation of superficial pellicle (case-hardening) may be avoided by pre-cooking, which considerably retards drying. It is clear that the more severe the initial conditions of cooking, the more stable is the subsequently dehydrated product. When an animal or plant is killed, its cells become more permeable to moisture as pointed out by Potter [131]. When the tissue is blanched or cooked, the cells may become still more permeable to moisture. Generally, cooked vegetable, meat, or fish is dried more easily than its fresh counterparts, provided cooking does not cause excessive shrinkage or toughening [131]. Cooking also results in a decrease in water-holding capacity of meat products [132].

27.7 CONCLUSION

Drying is a time-honored and energy-intensive method of food preservation. Thermal drying is divided into three types considering the atmosphere used in the drying system. These are air-drying, low-air-environment drying, and modified-atmosphere drying. Wide varieties of designs are available for each category. Significant efforts have been made to develop more energy-efficient drying systems considering improved dryer design as well as varied operating strategies. Different modes of energy, such as microwave, infrared, and radiofrequency, are being used to improve the process efficiency and quality of the dried products. It is also important to apply appropriate pre-treatments in order to improve product quality.

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28 Quality Changes during Drying of Foods

Mohammad Shafiur Rahman and Conrad O. Perera

CONTENTS

28.1 INTRODUCTION

Drying reduces the water activity, thus preserving foods by avoiding microbial growth and deteriorative chemical reactions. The heating effects on microorganisms and enzyme activity are also important in the drying of foods. In the case of foods to be preserved by drying, it is important to maximize microorganism and/or enzyme inactivation for preventing spoilage and enhanced safety, and to reduce the components causing dried foods' deterioration. On the other hand, in the case of drying bacterial cultures, enzymes, or vitamins, minimum inactivation of the microorganism and/or enzyme is required. Thus, the detrimental effects of drying may be desirable or undesirable depending on the purpose of the drying process. Initial freshness plays an important role in determining the quality of dried foods. The fresher the raw material, the better the stability and quality of the dried products. Suitable varieties or species of produce with desired maturity should be used to achieve the desired product with the best possible quality [1]. The quality characteristics of dried foods can be grouped as microbial, chemical, physical, and nutritional (Table 28.1).

28.2 MICROFLORA IN DRIED FOODS

Multiplication of microorganisms should not occur in properly processed dehydrated foods, but they are not immune to other types of food spoilage. If dried foods are safe in terms of pathogenic microbial count and toxic or chemical compounds, then acceptance depends on the flavor or aroma, color, appetizing appearance, texture, taste, and nutritional value of the product. Microbial standards are usually based on the total number of indicator organisms or the number of pathogens [2]. The microbial load and its changes during drying and storage are important information for establishing a standard that will ensure food safety. Perishable foods, such as meat and fish, are prone to rapid microbial spoilage, thus adequate care must be taken in drying. Poor processing, handling, and storage practices often result in a limited storage life of dried fish [3]. The microbial load for dried mackerel ranged from 3×10^3 colonies per gram sample to too numerous to count. No evidence of spoilage was detected in samples having water activity from 0.72 to 0.74. The isolates found were *Alcaligenes*, *Bacillus*, *Leuconostoc*, *Micrococcus*, *Halobacterium*, *Flavobacterium*, *Halococcus*, *Aspergillus*, and *Penicillium*. All the samples were positive for coliform,

TABLE 28.1
Quality Characteristics of Dried Foods

Microbial	Chemical	Physical	Nutritional
Pathogens	Browning	Rehydration	Vitamin loss
Spoiling	Oxidation	Solubility	Protein loss
Toxin	Color loss	Texture	Functionality loss
	Aroma development	Aroma loss	Fatty acid loss
	Removal of undesired components	Porosity	
		Shrinkage	
		Pores' characteristics	
		Crust formation	
		Structure	

Streptococcus, and Staphylococcus. *Vibrio* and *Clostridium* were not detected, while Salmonella was detected only in some samples [2]. Brining and drying decreased the microbial load but did not eliminate the pathogens. Wheeler et al. [3] studied the common fungi involved in spoiling of dried salted fish. They studied the mycoflora of dried salted fish with emphasis on visible spoiled fish and spoilage fungi. A total of 364 isolates from 74 fish were cultured and identified.

Wheeler and Hocking [4] studied the effect of water activity and storage temperature on the growth of fungi associated with dried salted fish. Microorganisms did grow during drying of highly perishable products such as fish (Trevally) in heat pump dehumidifier drying at low temperatures of 20°C to 40°C. Lower temperatures gave lower counts regardless of the relative humidity of drying. Sulfur-producing organisms were a significant portion of the total flora of fish drying. Rahman et al. [5] studied the endogenic microflora changes in tuna mince during convection air-drying between 40°C to 100°C. The drying temperature of 50°C or below showed no lethal effect on the microflora and showed significant growth. The drying temperature of fish must be above 60°C to avoid microbial risk in the product. The actual optimum temperature above 60°C should be determined based on other quality characteristics of the dried fish [5].

Reducing the water activity of a product inhibits growth, but does not result in a sterile product. The highest possible drying temperatures should be used to maximize thermal death even though low drying temperatures are best for maintaining organoleptic characteristics [6]. Another alternative is to use a high drying temperature initially at high moisture content and then drying at a low temperature. The microbial deactivation kinetics depends on several factors: variety, water content (i.e., water activity), temperature, and compositions of the medium (acidity, types of solids, pH, etc.) as well as the heating method [7–9]. Models to predict the decimal reduction times (D-values) were also developed as a function of temperature, pH, and water activity for isothermal conditions [10, 11]. These models could not be used in case of drying conditions since the level of water content does not remain same for each temperature studied. Bayrock and Ingledew [12] measured the D-values for the changing moisture content (i.e., drying) and for moist conditions (i.e., no change of moisture during heating). The heat resistance of microorganisms increased significantly during drying compared to the moist heat conditions. During drying of tuna, Rahman et al. [5] found that the D-value for endogenous microflora varied from 12.66 to 2.64 h when drying temperature varied from 60°C to 100°C, respectively. As expected the values were decreased with the increase of temperature, which indicates that an increase in drying temperature increased the lethal effect. The D-values at 100°C was much lower than the drying temperature at 90°C or below. This may be due to the high drying rate at 100°C [5, 13]. Rahman et al. [14] investigated the changes of endogenous bacterial counts in minced tuna during dry-heating (convection air-drying) and moist-heating (heating in a closed chamber) as a function of temperature. The D-values for total viable counts decreased from 2.52 to 0.26 h for moist heating and 2.57 to 0.34 h for dry-heating, respectively, when temperature was maintained constant within 60°C and 140°C. In both cases, increasing temperature caused significant decrease in D-values, whereas the effect of heating methods was not significant. The Z-values were 144°C and 46°C for temperatures within 60–100°C and 100–140°C, respectively. Rahman et al. [14] also identified the types and characteristics of endogenous microbes present in fresh and dried tuna. Initially tuna contained a mixture of different microbes, of which some are more heat- and/or osmo-tolerant than others. In dried tuna, the predominant microbes were moderately osmo-tolerant, and the dominant microbes were heat-sensitive.

Most of the vegetative cells of microorganisms will be destroyed by normal hot air-drying at 60–80°C with only a few exceptions (e.g., heat-resistant bacteria, yeast, and molds) [15]. Although there are some concerns about the potential for growth of microorganisms at the temperatures used in heat pump dryers, in practice there have been no reports of increased numbers of microorganisms in heat-pump-dried foods as compared to those dried by conventional means [16]. Serious microbiological problems may arise if the dryer is designed poorly.

Mold infections such as by *Aspergillus flavus* of fruits and vegetables could be a major source of contamination in dried products and can begin before harvest, especially under rainy and humid conditions, or during storage prior to drying or when these are damaged by insects [17]. Mold-infected products are usually removed during the sorting operation to prevent aflatoxin production and contamination that could make the product unsalable, since the maximum levels of aflatoxin allowed in nuts, grains, dried fruits, and milk are in the range of 0.5 to 15 parts per billion [18]. The best way to prevent fungal growth on harvested products is to maintain the optimum range of temperature and relative humidity throughout the handling system. Mold infection could also occur during the storage of products that have not reached a safe water activity level of 0.65 or less [17].

28.3 CHEMICAL CHANGES

28.3.1 Browning Reactions

Browning reactions change color, decrease nutritional value and solubility, create off-flavors, and induce textural changes. Browning reactions can be classified as enzymatic or nonenzymatic with the latter being more serious as far as drying processes are concerned. Two major types of nonenzymatic browning are and Maillard browning. In addition to the moisture level, temperature, pH, and the composition are all parameters that affect the rate of nonenzymatic browning. The rate of browning is the most rapid in the intermediate moisture range and decreases at very low and very high moistures. Browning tends to occur primarily in the middle of the drying period. This may be due to the migration of soluble constituents toward the central region. Browning is also more severe near the end of the drying period when the moisture level of the sample is low and less evaporative cooling is taking place, which occurs when the product temperature rises. Several suggestions are found to reduce browning during drying. In all cases, it was emphasized that the product should not experience unnecessary heat when it is in its critical moisture content range [6].

In meat products without nitrites, browning takes place during drying due to the denaturation of the globin protein in myoglobin [19]. Maillard-type nonenzymatic browning reactions in processed meat products also contribute to their external surface color. The main browning reaction involves the reaction of carbonyl compounds with amino groups, although lesser amounts of carbonyl browning also occur. Muscle usually contains small amounts of carbohydrates in the form of glycogen, reducing sugars and nucleotides, while the amino groups are readily available from the muscle proteins. Browning occurs at temperatures of 80–90°C and increases with time and temperature [20]. A loss of both amino acids and sugars from the tissue occurs because of the browning reaction. Lysine, hystidine, threonine, methionine, and cysteine are some of the amino acids that may become involved in browning [21]. Maillard browning proceeds most rapidly during drying when moisture content is decreased to a range of 15–20% [82]. As the moisture content drops further, the reaction rate slows so that in products dried below 2% moisture further color change is not perceptible even during subsequent storage. Drying systems or heating schedules generally are designed to dehydrate rapidly through the 15–20% moisture range to minimize the time for Maillard browning. In carbohydrate foods, browning can be controlled by removing or avoiding amines and conversely in protein foods by eliminating the reducing sugars. Ascorbic acid browning is another important browning reaction, which occurs when drying products rich in ascorbic acid. Green fruit and vegetables also turn brown during drying due to conversion of chlorophyll to pheophytin [17].

28.3.2 Lipid Oxidation

Dehydrated foods containing fats are prone to develop rancidity after a period, particularly if the water content is reduced too much. Fish oils or fats are more unsaturated than beef or butter, and they are usually classified as drying oils because they contain considerable proportions of highly unsaturated acids. The behavior of drying oils toward atmospheric oxygen is well known, and oxidation is a serious problem for commercial drying of fatty fish and seafood. The flesh of some fatty fish, such as herrings, contains a fat prooxidant that is not wholly inactivated by heat [22].

Lipid oxidation is responsible for rancidity, development of off-flavors, and the loss of fat-soluble vitamins and pigments in many foods, especially in dehydrated foods. Factors affected the oxidation rate include moisture content, type of substrate (fatty acid), extent of reaction, oxygen content, temperature, presence of metals, presence of natural antioxidants, enzyme activity, ultraviolet (UV) light, protein content, free amino acid content, and other chemical reactions. Moisture content plays a big part in the rate of oxidation. At water activities around the monolayer ($a_w \approx 0.3$), resistance to oxidation is greatest.

The elimination of oxygen from foods can reduce oxidation, but the oxygen concentration must be very low to have an effect. The effect of oxygen on lipid oxidation is also closely related to the product porosity. Freeze-dried foods are more susceptible to oxygen because of their high porosity. Air-dried foods tend to have less surface area and pores due to shrinkage, and are thus not affected by oxygen. Minimizing oxygen level during processing and storage, and the addition of antioxidants as well as sequestrants was recommended to prevent lipid oxidation [6]. Fish oils or fats are drying oils, which rapidly absorb oxygen from the air and harden just as paints harden on exposure to air. Fatty fish must be dehydrated quickly in a vacuum and must be stored in vacuum or in an atmosphere of an inert gas [23].

Antioxidants added to the herrings before drying are ineffective, but addition during drying with wood smoke, which contains simple antioxygenic phenols, stabilizes the fat of the dehydrated products considerably [22]. Oxidation of the fat normally occurs during dehydration. Herrings and haddock dried at 80°C to 90°C compared to lower temperatures were found more stable during storage [22]. One factor that may be important is the production of browning products from protein or nonfatty parts that gave antioxidant activity. The effectiveness of nonenzymatic browning products in preventing lipid oxidation was one of the mechanisms hypothesized by Karel [24] to prevent lipid oxidation.

The effect of water on the destruction of the protective food structure in some specific dehydrated foods is probably involved in the prevention of lipid oxidation in heated meat systems [24]. In systems in which there are both surface lipids and lipids encapsulated within a carbohydrate, polysaccharide, or protein matrix, the surface lipids oxidize readily when exposed to air. The encapsulated lipids, however, do not oxidize until the structure of the encapsulated matrix is modified and/or destroyed by adsorption of water [25]. Another reason is the increase of oxygen diffusion by increasing molecular mobility above glass–rubber transition [26].

The peroxide values of different dried meat samples were studied by Rahman et al. [27]. The values were significantly different according to the method of drying. Freeze-drying

gave the highest value, while air-drying gave the lowest value. Similar results were also observed in the case of air-dried, vacuum-dried, and freeze-dried tuna meat [28]. This was due to the increased oxygen diffusion and exposed surface area with the increase of porosity for freeze-dried samples [28].

Lowering the water activity by the removal of water brings about the extension of the shelf life of dried products, which almost always brings about some effect on the components of the dried products [17]. For example, drying retards the growth of bacteria, yeast, and molds. It can also result in lowering the enzymatic and chemical reaction rates. Dried products generally have greater stability, but water activity below the monomolecular layer could promote oxidation reactions.

28.3.3 Changes in Proteins

The protein matrix in muscle has a marked effect on its functionality and properties [29]. The nonfatty part of fish is very susceptible to changes caused by high temperatures during initial cooking, drying, and storage. Every process involved in the conversion of muscle to meat alters the characteristics of the structural elements [30]. Heating is believed to cause the denaturation of the muscle proteins even below 60°C, but not enough to affect much shear resistance [31]. The decrease in shear observed at 60°C was attributed to collagen shrinkage. Hardening at 70–75°C was believed to be due to increased cross-linking and water loss by the myofibrillar proteins, while decreasing shear at higher temperatures may indicate solubilization of collagen [20]. After 1 h at 50°C, the collagen fibrils of the endomysium appear beaded, which is brought about by their close association with the heat-denatured noncollagenous proteins in the extracellular spaces. Heat denaturation of the lipoprotein plasmalemma results at a temperature of 60°C for 1 h. The breakdown products of the plasmalemma are large granules and are often associated with the basement lamina, which appears to survive intact even after heating at 100°C for 1 h [32, 33]. Protein changes in relation to solubility, Maillard reaction, and protein cross-linking in whole milk powder during storage at different relative humidities were evaluated by Le et al. [34]. They found that the cross-linking of casein strongly influences the decrease in solubility of whole milk powder.

28.3.4 Volatile Development or Retention

In addition to the physical changes, drying generates flavor or releases flavor from the foods. Drying changes the composition of volatiles by evaporating most volatiles and by forming new volatile odor compounds by chemical reactions [35, 36]. Such changes in volatiles might affect the aroma of fresh foods after drying, for example, off-flavors were produced in peanuts when drying air temperatures were above 35°C. The amount of off-flavor detected in peanuts appeared to be a function of drying air temperature and moisture content, and the off-flavor was more likely to occur in immature peanuts than in mature peanuts [37]. Off-flavors resulting from high-temperature drying can be passed on to peanut butter and roasted peanuts. Acetaldehyde and ethyl acetate may be better indicators of off-flavor. Higher temperature drying of pasta was also related to off-color and off-flavor [38].

A substantial volatile loss occurred during the first three stages of spray drying, and there should be zero or very little loss of volatiles during the fourth stage due to selective diffusion [39]. Loses can occur during atomization, from undisturbed drops, and morphological development. Several factors affect volatile retention, including control of atomizer pressure or rotation speed, choice of spay angle, configuration of air input, alteration of air temperature profile, feed concentration, presence of an oil phase and/or suspended solids, foaming of the feed, feed composition, surfactant, and steam blanketing of the atomizer [39–41]. The retention increases with increasing initial concentration of solids, increasing air temperature and velocity, and decreasing humidity. This is due to the selective diffusion mechanism, when surface water content is reduced sufficiently so that the diffusion coefficients of volatile substances become substantially lower than that of water [40, 41].

There are potential improvements in the quality of heat-pump-dried products with optimum drying conditions in terms of a closed system with controlled temperature and humidity. Usually, dried products have low aroma volatile content, suffer a loss of heat-labile vitamins, and have a high incidence of color degradation. Ginger dried in a heat pump dryer was found to retain over 26% of gingerol, the principal volatile flavor component responsible for its pungency, compared to only about 20% in rotary-dried commercial samples [42]. The higher volatile retention in heat-pump-dried samples may be due to reduced degradation of gingerol at the lower drying temperature used compared with the commercial dryer temperatures. The loss of volatiles varies with concentration, with the greatest loss occurring during the early stages of drying when the initial concentration of the volatile components is low. Since heat pump drying is conducted in a sealed chamber, any compound that volatilizes remains within it, and the partial pressure for that compound gradually builds up within the chamber, retarding further volatilization from the product [43].

28.3.5 Color Retention or Development

High temperature and long drying time degrade a product's original color. Color in foods can be preserved by minimal heat exposure or applying high temperature and short time with pH adjustment. Water activity is one of the important factors degrading chlorophyll. Another cause of color degradation may be due to enzymatic browning causing rapid darkening, mainly of the leafy portions. The formation of dark pigments via enzymatic browning is initiated by the enzyme polyphenol oxidase (PPO). Another reason for discoloration is photooxidation of pigments, caused by light in combination with oxygen.

Development of a brown center sometimes occurs in macadamia nuts if high-moisture nuts are dried at elevated temperatures [44]. Heat pump drying of macadamia nuts did not result in brown centers, even when they were dried at 50°C [45]. Mason [46] studied the heat pump drying of macadamia kernels and herbs with temperature and relative humidity ranges of 30–50°C and 0.10–0.50, respectively. Freshly harvested macadamia nuts can be dried rapidly up to a moisture content of 0.015 with no loss in quality, and there was no significant difference of quality when dried under the aforementioned conditions. This may be due to the faster drying rates in the heat pump drying process [43]. The losses in color, flavor, and nutritive value associated with dried products are attributed to nonenzymatic browning. It is recognized that the rate of reaction for nonenzymatic browning in dried products is highest at moisture levels that are commonly attained toward the end of the drying cycle, when the drying rate is low and the product temperature approaches that of the drying medium. However, the lower drying temperatures used throughout the drying cycle in heat pump dryers reduce the extent of nonenzymatic browning reactions.

The color and aroma of herbs (e.g., parsley, rosemary, and sweet fennel) can be improved when compared with commercial products. The sensory values were nearly doubled in the case of heat-pump-dried herbs as compared to commercially dried products. There was no significant difference in the quality of herbs dried below moisture content 0.04 for the experimental drying temperatures (40°C and 50°C) and relative humidity (0.30 and 0.40). A wide range of quality characteristics can be obtained by running the dryer within wide ranges of temperature and relative humidity. The use of modified atmospheres for drying of sensitive materials such as food products is another important potential aspect of heat pump drying technology. During drying, oxygen-sensitive materials, such as flavor compounds and fatty acids, can undergo oxidation, giving rise to poor flavor, color, and rehydration properties. Using modified atmospheres to replace air could permit development of new dry products without oxidative reactions [43].

Technologies to create modified-atmosphere (MA) drying are now evolving. Apple tissues dried by modified-atmosphere heat pump (MA–HP) drying had lighter color, lower bulk density values, porous (noncollapsed) structure, and better rehydration properties, as compared to those dried by most other common drying methods [47]. O'Neill et al. [48] showed that browning of apple cubes during drying could be arrested when the oxygen level in the atmosphere is less than 0.5%. Apple cubes dried in nitrogen atmosphere gave more open pores and uniform shrinkage than those dried in air and vacuum. Rahman et al. [27] studied the microbial (aerobic plate count, *Pseudomonas*, *Staphylococcus*, molds) and physicochemical (pH, expressed juice, fatty acid profile, rehydration ratio, color) characteristics of sun, air, vacuum, freeze, and modified atmosphere (nitrogen gas) dried goat meat. The modified atmosphere drying showed significant improvement in selected quality attributes, such as shrinkage, color, types of molds, and peroxide values.

28.4 PHYSICAL CHANGES

28.4.1 Structural Changes

Structural changes in food during drying are usually studied by microscopy. It provides a good tool to study this type of phenomena as well as other types of physical and chemical changes during the drying of food materials. Shrinkage occurs first at the surface and then gradually moves deeper into the food with increase in drying time [49]. The cell walls became elongated. As drying proceeds at higher temperatures, cracks are formed in the inner structure. From microscopy, it was found that the shrinkage of apple samples dried by convection is significantly anisotropic, while less damage to the cell structure during freeze-drying leads to a more isotropic deformation [50]. The cellular structure of microwave-vacuum dried apple with and without osmotic treatment indicated collapse of the cellular structure in the untreated apple [51]. Osmotic treatment prior to vacuum drying preserved the cellular structure by retaining the three-dimensional nature. Electron microscopic investigations of the cell structure in dried carrots and green bean showed that drying leads to shrinkage and twisting of the cells and clumping of the cytoplasm [52]. Histological changes in air-dried, freeze-dried, and osmotically treated freeze-dried samples showed that air-dried samples showed the elongated and thinned cell wall and enlarged intercellular air spaces [53].

Heating produces major changes in muscle structure. Voyle [54] reviewed modifications in cooked tissue observable with a scanning electron microscope. Alternation in muscle structure due to heating include coagulation of the perimysial and endomysial connective tissue, sarcomere shortening, myofibrillar fragmentation, and coagulation of sarcoplasmic proteins [54, 55]. Heating and/or drying intensifies the detachment of the myofibrils from the muscle fiber bundles, which is caused mainly by electrical stunning or stimulation, and improper conditioning following slaughter [56].

Rehydration is maximized when cellular and structural disruption such as shrinkage is minimized [6]. Chang et al. [57] illustrated the morphological changes that occur in the appearance of the muscle fiber bundles during cooking and drying in a convection-heated rotary dryer. They found that after cooking the fibers are bound together in a compact bundle. The bundle size is gradually reduced due to the effects of heating and tumbling during the early stage of predrying in the modified clothes dryer. Apparent bundle size is expanded with the endomysial capillary moisture being removed during drying.

28.4.2 Case Hardening or Crust Formation

During drying, the concentration of moisture in the outer layers is less than in the interior, since outer layers necessarily lose moisture before the interior. This surface shrinkage causes checking, cracking, and warping. This type of shrinkage causes moisture gradient and resistance near the surface. In extreme cases, shrinkage and drop in diffusivity

may combine to produce a skin practically impervious to moisture, which encloses the volume of the material so that interior moisture cannot be removed. This is called case hardening. In food processing, case hardening is also commonly known as crust formation. Extension of crust formation can be reduced by maintaining flattening moisture gradients in the solid, which is a function of drying rate. The faster the drying rate, the thinner the crust [58]. Crust (or shell) formation may be either desirable or undesirable in dried food products. In microencapsulation of flavors, rapid crust formation is required to prevent flavor losses. Achanta and Okos [58] pointed that crust formation may be inhibited by allowing the drying rate to be slow enough that moisture loss from the product surface is replenished by moisture from inside. Crust formation is also important in explosion puffing. In this case the high-moisture product is exposed to rapid drying conditions such as high temperature and vacuum, which create crust. The impermeable crust coupled with the extreme drying conditions results in rapid moisture vaporization and causes large internal pressures to build up, resulting in product expansion/puffing. During the expansion stage, stress buildup in the glassy surface may cause the surface to crack, allowing vapor to escape.

28.4.3 Shrinkage or Collapse and Pore Formation

Two types of shrinkage are usually observed in food materials: isotropic and anisotropic. Isotropic shrinkage can be described as the uniform shrinkage in all geometric dimensions of the materials. Anisotropic shrinkage is described as the nonuniform shrinkage in the different geometric dimensions. In many cases, it is important to estimate the changes in all characteristic geometric dimensions to characterize a material. In fish and seafood shrinkage in the direction parallel to muscle fibers was significantly different from that perpendicular to the fibers during air-drying [59, 60]. This is different from the very isotropic shrinkage of most fruits and vegetables.

Shrinkage is an important phenomenon, which impacts the quality of dried food product by reducing product wettability, changing product texture, and decreasing product absorbency. Depending on the end-use, crust and pore formation may be desirable or undesirable. If a long bowl life is required for a cereal product, a crust product that prevents moisture reabsorption may be preferred. If a product (such as dried vegetables in instant noodles) with good rehydration capacity is required, it should be highly porous with no crust. Rahman [61] provides the present knowledge on the mechanism of pore formation in foods during drying and related processes. The glass transition theory is one of the proposed concepts to explain the process of shrinkage and collapse during drying and other related processes. According to this concept, there is negligible collapse (more pores) in material if processed below glass transition, and the higher the difference between the process temperature and the glass transition temperature, the higher the collapse. The methods of freeze-drying and hot air drying can be compared based on this theory. In freeze-drying, since the temperature of drying is below T_g' (the maximally freeze concentrated glass transition temperature), the material is in the glassy state. Hence, shrinkage is negligible. As a result the final product is very porous. In hot air drying, on the other hand, since the temperature of drying is above T_g' or T_g, the material is in the rubbery state and substantial shrinkage occurs. Hence, the food produced from hot air drying is dense and shriveled [58]. However, the glass transition theory does not hold true for all products. Other concepts such as surface tension, structure, environment pressure, and mechanisms of moisture transport also play important roles in explaining the formation of pores. Rahman [61] hypothesized that as capillary force is the main force responsible for collapse, counterbalancing of this force causes formation of pores and lower shrinkage. The counterbalancing forces are due to generation of internal pressure, variation in moisture transport mechanism, and environmental pressure. Another factor could be strength of the solid matrix (i.e., ice formation, case hardening, and matrix reinforcement).

28.4.4 Stress Development and Cracking or Breakage

During air-drying, stresses are formed due to nonuniform shrinkage resulting from nonuniform moisture and/or temperature distributions. This may lead to stress crack formation, when stresses exceed a critical level. Crack formation is a complex process influenced interactively by heat and moisture transfer, physical properties, and operational conditions [62]. Air relative humidity and temperature are the most influential parameters needed to be controlled to eliminate the formation of cracking.

The cracking and breakage of dried foods have two undesirable consequences: loss of valuable product and loss of consumer satisfaction [58]. Cracking is detrimental to grain quality since affected kernels are more susceptible to mold attack during storage and pathogenic invasion after seeding. Cracked grains are also of lower organoleptic quality, which limits their use in direct food preparation. Internal cracking in the starchy endosperm of a grain is induced by mechanical stress due to the high humidity gradient inside the kernel and/or to thermal stress. The fissure is a large internal fracture usually found to be perpendicular to the long axis of grain [63]. The drying rate, which is a function of drying temperature and humidity, is the main cause of fissures [64–66]. The process of fissures also continues after drying. Most fissuring occurs within 48 hours after drying, but additional fissures develop at a low rate for another 72 hours thereafter [67]. In microwave drying, stress cracking can be even more pronounced due to superposition of the pressure gradient that may build up within the material under certain drying conditions [83]. In case of wheat it also depends on the variety [68]. High-humidity air damages grains to a lesser extent than low-humidity air. Grains are severely damaged by high drying temperatures [69]. The effect of drying air temperature on the mechanical properties of corn kernels was investigated by Abasi and Minaei [70], who found that an increase in drying

temperature from 40°C to 70°C increased kernel deformation at the rupture point by an average of 12%.

In the case of plant materials, cracks also formed. At higher drying rates the outer layers of the material become rigid and their final volume is fixed early in the drying. As drying proceeds, the tissues split and rupture internally forming an open structure, and cracks are formed in the inner structure. When the interior finally dries and shrinks, the internal stresses pull the tissue apart [49]. The initial structure before drying can also create different types of cracks inside as well as on the surface.

28.4.5 Rehydration

Rehydration is a process of moistening dry material. It is usually done with an abundant amount of water. In most cases, dried foods are soaked in water before cooking or consumption, thus rehydration is one of the important quality criteria. In practice, most changes during drying are irreversible and rehydration cannot be considered simply as a process reversible to dehydration [71]. In general, absorption water is fast at the beginning and thereafter slows down. Rapid moisture uptake is due to surface and capillary suction. Rahman and Perera [72] and Lewicki [71] reviewed the factors affecting the rehydration process. These factors are porosity, capillaries and cavities near the surface, temperature, trapped air bubbles, amorphous-crystalline state, soluble solids, dryness, anions, and pH of soaking water. Porosity and capillaries and cavities near the surface enhance the rehydration process, whereas the presence of trapped air bubbles is a major obstacle to the invasion of fluid. Until the cavities are filled with air, water penetrates the material through its solid phase. In general, temperature strongly increases the early stages of water rehydration. The resistance of crystalline structures to solvation causes development of swelling stresses in the material, whereas amorphous regions hydrate fast. The presence of anions in water affects volume increase during water absorption.

28.4.6 Solubility

Many factors affect solubility, including processing conditions, storage conditions, composition, pH, density, and particle size. It was found that increased drying temperatures are accompanied by increasing protein denaturation, which decreases solubility. Thus, more protein is denatured and solubility decreased [6]. Removal of water by evaporation results in formation of an amorphous state. Le at al. [34] found that the cross-linking of casein strongly influences the decrease in solubility of whole milk powder.

28.4.7 Caking and Stickiness

Caking and stickiness of powders, desirable or undesirable, occur in dried products. Caking is desirable for tablet formation and undesirable when a dry, free-flowing material is required. To reduce caking during drying, a logical option is to dry rapidly so that moisture content drops to a level where caking is inhibited. The rapid drying will form a crust, which may be undesirable, thus product optimization or solutes in product formulation may be considered. Tendencies to form surface folds on particles during spray drying are governed by the viscosity of the concentrated solution. Stickiness and agglomeration tendencies also depend upon the viscosity of the concentrated solution, surface tension, particle size, and exposure time [41]. For viscosities below the critical value, stickiness usually occurs. The predicted critical viscosity was within the range of 10^8–10^{10} Pa s. The mechanism of sticking and agglomeration was postulated through viscous flow driven by surface tension and forming bridges between particles [73]. Adhikari et al. [74] presented a complete review of stickiness in foods including mechanisms and factors controlling the process. The main factors affecting stickiness are temperature, viscosity, and water followed by low molecular sugars, organic acids, and compaction or pressure. The use of a model based on glass transition temperature provides a rational basis for understanding and characterizing the stickiness of many foods. Much scientific progress has been made recently in understanding the physical phenomena surrounding stickiness. They involve combining the science of soft matter, along with molecular dynamics simulation and/or particle interaction simulation based on physical principles, and various approaches to the measurement of stickiness and related aspects of glass transition [75].

28.4.8 Texture

Factors that affect texture include moisture content, composition, variety or species, pH, product history (maturation or age), and sample dimensions. Texture is also dependent on the method of dehydration and pretreatments. Purslow [76] stated that meat texture is affected by the structure of the solid matrix. He concluded that it is important to have a fundamental understanding of the fracture behavior of meat and how it relates to the structure of the material. Stanley [30] stated that many researchers now believe the major structural factors affecting meat texture are associated with connective tissues and myofibrillar proteins. Moreover, two other components—muscle membranes and water—also deserve consideration not because of their inherent physical properties but rather as a result of the indirect influence they have on the physical properties. It should be noted that sarcoplasmic proteins could be important for the same reason, although little information on their role is available. He suggested that these structures merit particular attention.

Kuprianoff [77] referred to the possible adverse effects of removing bound water from foods: (i) denaturation of protein by concentration of the solutes, (ii) irreversible structural changes leading to textural modification upon rehydration, and (iii) storage stability problems. Stanley [30] stated that the water-holding capacity of muscle is related to its sorption properties. The bound water in the muscle is primarily a result of its association with the myofibrillar proteins as indicated by Wismer-Pedersen [78]. Protein–water interactions

significantly affect the physical properties of meat [79]. Changes in water-holding capacity are closely related to pH and to the nature of muscle proteins.

28.5 NUTRITIONAL CHANGES

28.5.1 Vitamin Retention

In general, losses of B vitamins are usually less than 10% in dried foods. Dried foods do not greatly contribute to dietary requirements for thiamin, folic acid, and vitamin B-6. Although vitamin C is largely destroyed during drying due to heating, meat per se is not a good source [20]. From non-fatty vegetables, such as cabbage, as much water as possible should be removed, because this helps to conserve ascorbic acid. The loss of vitamin A and ascorbic acid in dried products could be avoided in the absence of oxygen. Even though most amino acids are fairly resistant to heating–drying, lysine is quite heat-labile and likely to be borderline or low in the diet of humans and especially so in developing countries where high-quality animal proteins are scarce and expensive [80]. The fruits dried by MA–HP drying retained the highest level of nutrients, such as vitamin C and flavor compounds [43]. These observations suggest that MA–HP is highly suited for the drying of sensitive food and pharmaceutical products.

28.5.2 Polyphenol Retention

In the case of blueberries, freeze-drying resulted in higher retention of total polyphenols and anthocyanins, and these are comparable to hot air microwave vacuum drying. Similarly, freeze-drying retained higher antioxidant activity followed by hot air microwave vacuum drying and hot air drying. Aglycone polyphenols are more stable as compared to glycosides under dehydration conditions [81].

28.6 CONCLUSION

Drying is a process of water removal that results in reduced water activity and that can cause microbial destruction, prevent microbial growth, reduce deteriorative chemical reactions, and control physical changes. These changes in dried foods depend on the drying methods and their operating conditions as well as pretreatments used. The most important factors are temperature, pressure, atmosphere of drying, and electro-energy used. In addition, the types of foods, and their compositions and structure play a role in achieving desired quality of the dried foods.

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29 Smoking and Food Preservation

Mohammad Shafiur Rahman and Kutaila Al-Farsi

CONTENTS

29.1 INTRODUCTION

Smoking of foods is one of the most ancient and traditional food preserving processes, and in some communities one of the most important. The use of wood smoke to preserve foods is as old as open-air drying. Smoking is a slow process and it is not easy to control as a drying process. Although it is not primarily used to reduce the moisture content of food, the heat associated with the generation of smoke also causes drying. Smoking has been mainly used with meat and fish [1]. It also can be applied to chicken [2], mushrooms [3], and cheese [4, 5]. The degree of freshness and proper handling and storage conditions play a role in achieving a good quality smoked product. Presmoking treatments, such as splitting, salting, and hanging, are also used, depending on the type of product [1].

29.2 PURPOSE OF SMOKING

The main purposes of smoking are it imparts desirable sensory flavors and colors to the foods, it inactivates enzymatic compounds and microorganisms, and some of the compounds formed during smoking have preservative effects (i.e., bactericidal and antioxidant) due to presence of a number of antioxidant and antimicrobial compounds [1, 6, 7]. In many cases, smoking is considered as a pretreatment rather than a drying process. It was found that smoke is effective in preventing lipid oxidation in meat and fish products [8]. Smoke deposition is effective only in controlling surface spoilage [9]. Smoking technology is used in the food industry to enrich meat products for a desired organoleptic profile [10, 11]. It can effectively preserve white fish for a longer time as compared to fatty fish [12].

29.3 SMOKING PROCESSES

Smoking is a slow process and it is not easy to control. Smoke contains phenolic compounds, acids, and carbonyls, and smoke flavor is primarily due to the volatile phenolic compound [13, 14]. Wood smoke is extremely complex and more than 400 volatiles have been identified [15, 16]. The smoke generated depends on the type of generator, wood type, humidity, and temperature of the smokehouse [17]. Polycyclic aromatic hydrocarbons are ubiquitous in the environment as pyrolysis products of organic matter, and its concentrations in smoked food could reach levels hazardous for human health, especially when the smoking procedure is carried out under uncontrolled conditions [18]. Wood smoke contains nitrogen oxides, polycyclic aromatic hydrocarbons, phenolic compounds, furans, carbonylic compounds, aliphatic carboxylic acids, tar compounds, carbohydrates, pyrocatechol, pyrogallols, organic acids, bases, and carcinogenic compounds like 3:4 benzpyrene. Nitrogen oxides are responsible for the characteristic color of smoked food, whereas polycyclic aromatic hydrocarbon components and phenolic compounds contribute to its unique taste. These three chemicals are also the most controversial from a health perspective [16]. The level of fats in fish affects texture, oiliness, and color of smoked salmon during storage [13, 19].

It is important to use processing conditions, which must be standardized, controlled, monitored, and documented so that the potential of toxic-component generation in food products is eliminated or reduced. This is especially true for seafood products, which may contain food poisoning organisms of marine origin that are more difficult to control than land sources [9]. Color development in smoked fish is a complex process. Maillard type with glycolic aldehyde and methylglyoxal in the dispense phase of smoke play a dominant role. Several types of synthetic colors, paprika, caramel, and seasoning can also be used [20].

29.3.1 Cold Smoking

Cold smoking is defined as the smoking of food at low temperatures (i.e., below 33°C). Cold-smoked food is neither cooked nor protein coagulated in the process and it needs additional preservation hurdles, which include thermal processing, vacuum packaging, salting, and chilled storage. The main steps

of cold smoking are salting, drying, and smoking at temperature lower or equal to 30°C [18]. Several studies investigated the effect of these methods on the final product's properties and compositions. Indrasena et al. [21] studied the effect of cold smoking and drying on the textural properties of farmed Atlantic salmon. Atlantic salmon fillets were brine-salted at concentrations ranging between 2.2% and 3.6%, chilled, and cold smoked for different times. They found that the moisture content varied inversely with salt and it decreased with the increase of salt content. The breaking strength increased with the decrease in moisture.

Birkeland et al. [22] investigated the effect of process parameters (i.e., brine injection pressure, number of repeated injections, needle speed, injection of brine in one or two directions, and resting the chilled fillet before smoking) on the final product yield. They found that repeated injections increased the yield up to 5.3% (w/w) and the increase of injection pressure increased the fillet gaping by 18%. Birkeland et al. [23] also studied the quality parameters of cold-smoked fillets of Atlantic salmon using two different cold smoke methods: gentle cold smoking (i.e., manual filleting and dry salting) and tough cold smoking (i.e., machine filleting, injection salting, extended drying). They found that the product yield, water content, and gaping score were high and softer in texture in tough cold smoking than gentle cold smoking. However, the quality traits of the smoked products were difficult to predict from raw material characteristics. It is difficult to consistently retain the quality traits due to differences in color, texture, gaping scores, and total liquid loss of raw material. The stability of a smoked fish depends on the choice of processing method and processing hurdles used to preserve the product.

Birkeland and Skara [24] studied two different methods to produce cold smoke: liquid smoke protocol (SCP) and wood chips protocol (WCP). Salmon fillets were dry salted (18 h, 4°C) before being smoked by SCP or WCP. SCP included drenching for 1 min in smoke condensate (1:3 smoke:water) and drying for 150 min at 28.4°C ± 2.2°C. WCP included drying and smoking in a smoking chamber at 23°C for 480 min using wood chips for smoke production. The quality of cold-smoked salmon was assessed at 0, 7, 14, and 31 days of storage at 3.4°C. The SCP procedure resulted in a significantly higher processing yield (89.6%) compared to the WCP (88.6%). At the beginning, the SCP fillets showed light and yellow color and had a lower chroma (color C*) and hue (color h*) as compared to the fillets processed with WCP, but after one week the color differences were very small. Texture profile analysis (TPA) showed significant differences between the processing protocols, but the SCP fillets were significantly softer than the WCP fillets. The SPC or drenching method could be used to produce cold-smoked salmon with quality characteristics similar to traditional products.

The temperature used in cold smoking is important and could be a critical factor that can affect the final characteristics of the smoked product. Hultmann et al. [25] investigated the effect of smoking temperature (21.5°C, 24.3°C, 28.2°C, and 29.9°C, for 6.5 h) on different properties of proteins and enzymes in cold-smoked salmon. The smoking temperature was important for the protein solubility properties and myofibrillar protein composition in smoked salmon. The increase in smoking temperature caused a reduction in the extractability of myofibrillar proteins, and free amino acid content was increased with the increase of smoking time and storage duration. However, smoking temperature did not affect the composition of free amino acids as well as the proteolytic activity, and the effect of processing parameters was mostly observed at the early stages of the product's shelf life.

Fish are highly perishable and can easily spoil after a short time. Cold smoking could be used as a method to add value to fish products. Gomez-Guillen et al. [26] studied the suitability of sardine (i.e., lean and fatty), dolphin fish, and blue whiting for cold smoking. The samples were assessed by sensory, chemical, and microbial analysis over the storage time. They found that cold smoking could be used to add value to sardine and dolphin fish catches, if these are in excess. The water-holding capacity was stable for some species, and rancid flavors were only detected in sardine at the end of the storage period. In terms of microflora, lactic acid bacteria were dominant and Enterobacteriaceae did not show any growth. However, the blue whiting was found unsuitable for smoking due to softening of muscle tissue and the development of bitter taste and off-flavors. However, smoking of fish could add value to fish and decrease fish waste due to spoilage.

It was reported that the cold-smoked fishes had the highest prevalence of bacteria among other minimally processed foods [27]. The application of cold smoking does not apply severe stress to kill pathogens such as *Listeria monocytogenes*, and this is due to the application of low temperature (<30°C), salt (3–5%), and low storage temperature [28]. Therefore, there is a need to add other hurdles to inhibit the growth of microorganisms. *Listeria monocytogenes* is the most common microorganism found in cold-smoked foods. All smoked fish must be stored in chilled conditions or vacuum packed to prolong shelf life. Hansen et al. [29] identified the microflora on spoiled, sliced, and vacuum-packed cold-smoked salmon from three different sources. Lactic acid bacteria dominated, with large numbers of *Enterobacteriaceae*. The microflora on cold-smoked salmon appeared to be related to the sources of contamination, i.e., the raw material and/or the smokehouse.

Neunlist et al. [30] studied the effect of different steps of the cold smoking process and vacuum storage on the culture ability and viability of *Listeria monocytogenes* inoculated in sterile salmon samples. Salmon portions were inoculated with *Listeria monocytogenes* at a level of 6 log cfu/g, then salted (5.9%), smoked, partially frozen, vacuum packed, and stored for 10 days at 4°C followed by 18 days at 8°C. Neunlist et al. found that salting was the only step that had a significant effect on *Listeria monocytogenes* with 0.6 log reduction; other processing steps did not show any immediate effect. However, the combination of steps significantly lowered *Listeria monocytogenes* by 1.6 log cfu/g, and the count remained less than 7 log cfu/g until the end of the storage period, whereas unprocessed (control) samples reached up to 9 log cfu/g. This indicates that there is a need to add other hurdles to preserve the cold-smoked product.

Nykanen et al. [27] studied the inhibition effect of nisin and sodium lactate or their combination on *Listeria monocytogenes*, and mesophilic aerobic bacteria in cold-smoked rainbow trout. Nisin of 4000–6000 IU/ml, sodium lactate of 60%, and a combination of both substances (1:1) were injected into rainbow trout fish before the smoking process, or injected in the finished smoked product. A *Listeria monocytogenes* level of 10^3–10^4 cfu/g was inoculated in the fish samples. All samples were packed under vacuum and stored at 8°C for 17 days or at 3°C for 29 days. Both nisin and lactate inhibited the growth of *Listeria monocytogenes* in smoked fish, but the combination of nisin and sodium lactate decreased the count from 3.3 to 1.8 log cfu/g at 8°C for 16 days of storage (i.e., autosterilization occurs to some extent). These preservatives showed no effect on sensory properties of the product until a period of 23 days stored at 3°C.

29.3.2 Hot Smoking

The traditional method of smoking fish uses hot smoke, from a range of woods, passed over the fish to partially dry it and impart the flavor and aroma of the smoke. The disadvantages of this method include a lack of control over the process and health concerns if the surface of the fish is not properly dried. The smoking process involves extensive handling of raw and finished products. Smoked food is prepared with two basic procedures. One cooks the product (hot smoking) and the other does not (cold smoking). Cold-smoking devices have one basic function: to apply smoke to the product. Hot-smoking devices provide the added function of applying heat. The hot-smoke process for smoking fish differs from the cold-smoke process in a fundamental way. The cold-smoke process requires that the fish reaches an internal cooking temperature below 35°C, while the hot-smoke process cooks the fish center to at least 62.8°C for at least 30 minutes, and fish must contain at least 3.5% water phase salt for both processes [9]. A traditional wood-burning kiln has a temperature of between 300°C and 700°C while an oven's temperature is usually above 80°C [31]. A temperature between 4°C to 61°C (i.e. risk zone) can create an environment favorable to bacteria growth. As an additional safety margin, hot-smoked fish should always be cooled to less than 3.3°C immediately after smoking and held at that temperature until consumed. Both hot- and cold-smoked fish are preserved primarily by controlling salt (i.e., water phase salt), moisture content, and chilled condition.

The hot smoking of fish requires five steps, each one with different goals and operating conditions. These steps are surface drying, smoking, drying, heating/cooking, and cooling. Surface drying is the removal of surface moisture leaving a protein coating (pellicle) on each piece of fish and adsorbing an even deposition of smoke. A dense smoke atmosphere, where smoke is deposited evenly on the surface of each piece, is important and it ensures good flavor, color, and surface preservation. Often color does not develop until the surface of the fish reaches 54.4°C to 60°C during the cooking step. Evenly drying the fish is needed to reduce moisture, and raises salt in the water phase and establishes final texture. This is a critical step in producing safe products. It is required to heat each piece of fish to at least 62.8°C and to hold that temperature for at least 30 min. This is also a critical step in producing safe smoked fish. Cooling the fish below cooking temperature (48.9–60°C) in the smokehouse as quickly as possible is needed. A suitable sanitary refrigerated room is usually more practical and cost-effective than a refrigerated smokehouse. Cold-smoke procedures do not use step 4 of heating/cooking. Usually these five cycles take 8–12 hours. Cycles of 4 hours or less are possible with thin and lightly smoked products [9]. The differences in the process employed depend primarily upon the type of fish and regional preferences for a particular product.

Hot smoking is preferred because it extends the shelf life of food products. Hot smoking uses a thermal process, which combines heating and drying with smoking, leading to the creation of unfavorable conditions for the growth of harmful microorganisms. However, processing at high temperatures is considered responsible for the qualitative degradation of processed products. Thus, several techniques are required to minimally heat the hot-smoked products [1, 32]. Moreover, the rate of deposition of volatile compounds depends on temperature, moisture, flow rate, and density of the smoke; the water solubility and volatility of the particular compounds; and the properties of the fish surface [33].

Fafioye et al. [34] studied the fungal infestation of five traditionally smoked dried freshwater fish. They isolated and identified 11 different fungal species, and *Aspergillus flavus* was the most frequently encountered fungi on the fish species. Adebayo-Tayo et al. [35] reported the presence of aflatoxin and other metabolites due to *Aspergillus flavus* in smoked fish sold in Nigeria and confirmed that consumers could have been at risk of aflatoxin poisoning.

Smoking methods (traditional drum and convective kilns) affected the proximate composition, quality, and safety (heavy metals and microbial) of traditional smoked fish (i.e., five types) [36]. In the case of traditional drum kiln, the fish was skewed, salted, and smoked to 60–90°C for 36 hours. The fish for convective kiln were cut into uniform pieces (fillet) so that no parts would get overheated, and cooked to an internal temperature of 80°C (checked by thermocouple) for 12 hours. The moisture content of fresh fish samples ranged from 73.5% to 75.9%, and drum-smoked fish samples ranged from 11.9–13.4% and 8.6–9.4% for convective-smoked fish samples. The proximate composition, rancidity indices (i.e., peroxide value [PV], thiobarbituric acid [TBA], total volatile base-nitrogen [TVB-N], trimethyl amine value [TMA]), fatty acids, and pH varied with the types of fish. Four heavy metals (i.e., lead, cadmium, mercury, and chromium) investigated in the fish samples were generally below the maximum permissible levels set by the World Health Organization (WHO). Fresh fish showed the presence of *Listeria monocytogenes* (1.2×10^2–1.7×10^2 cfu/g), *Staphylococcus aureus* ($1.2–1.7\times10^2$ cfu/g), *Salmonella paratyphi* ($1.0–1.5\times10^2$ cfu/g), and *Escherichia coli* ($1.3–1.6\times10^2$ cfu/g), and the total viable count varied from $8.4–9.2\times10^8$ cfu/g. The convective and drum smoked showed total viable count varied from $8.4–9.2\times10^2$ cfu/g and

2.3–6.4×10^4 cfu/g. The convective-smoked fish showed the absence of *Listeria monocytogenes*, *Salmonella paratyphi*, and *Escherichia coli*, while *Staphylococcus aureus* varied from absent to 2.2 × 10^2 cfu/g. The drum-smoked fish showed absence of *Salmonella paratyphi* and *Escherichia coli*, but the presence of *Listeria monocytogenes* (4.4–12.2×10^1 cfu/g) and *Staphylococcus aureus* (2.1–5.7×10^2 cfu/g). Traditional drum-smoked fish may pose higher microbiological risks due to the presence of *Listeria monocytogenes*.

A hybrid fish-smoking kiln that uses propane gas as a source of heat in combination with biomass (i.e., sawdust, rice husk, and wood) was successfully designed and fabricated [37]. The smoking temperature of 120°C (i.e., maximum recommended) was reached in 2 h when 1 kg of each biomass was used in combination with propane gas, and the temperature dropped to 60°C in 1 h when the propane gas was switched off. Similar weight loss, proximate composition, smoke density, phenol content, and sensory qualities of the smoked fish were obtained for the three biomasses used. Catfish smoked in the hybrid kiln were generally acceptable. The hybrid kiln yielded quality smoked fish in terms of phenol content, biomass utilization, reduced smoking time, reduced cost of production (low wood consumption, smoke dusting, and drying rate), and uniform product.

After hot smoking, the smoked product is stored at chilled or refrigerated conditions for about a month [38]. In order to increase the shelf life of hot-smoked products, they should be stored at very low temperatures. In fact, hot-smoked foods can be preserved at –30°C for more than 6 months. Preservatives are used in order to increase the shelf life of hot-smoked food products. Coban et al. [39] investigated the effects of some oils on the chemical, microbiological, and sensory quality of smoked rainbow trout fillets packed in vacuum. They found that the essential oils (natural antioxidants) can be used in vacuum packs to enhance hot-smoked fish quality and increase the shelf life of the products up to 112 days. Other preservatives including chitosan, sodium lactate, sodium diacetate [40], thyme and garlic oil [12], and essential oils [41, 42] to extend the shelf life of vacuum-packed hot-smoked products. Da Silva et al. [43] examined the microbial safety and quality of smoked blue catfish steaks treated with antimicrobials and antioxidants during 6 weeks of ambient storage.

In addition, different processing schemes are specified for different fish species [13]. The smokehouse is equipped with a smoke generator where smoke is passed over water to remove tar and solid particles. Good Manufacturing Practices (GMP) from the U.S. Food and Drug Administration (FDA) sets minimum standards for time/temperature smoking cycles, salt and moisture content, manufacturing procedures, holding and shipping temperatures, process monitoring and record keeping, and packaging.

29.3.3 Liquid Smoking

Modern methods of smoked fish use formulated liquid smoke and these provide flavor with reduced water activity on the surface. In this method, the fish are dipped in smoke solutions prior to drying. Most drying methods use heat to change the relative humidity of the air passing over the fish. This is an inefficient use of energy and the heat drives off many of the aromatic chemicals that go to make up the aroma, flavor, and color of the product. This could be overcome using an energy-efficient heat pump drier, where drying is performed in a closed chamber.

Smoke solutions are available, either being condensed products from the dry distillation of wood or synthetically prepared mixtures of phenols. The use of smoke condensates offers some advantages. These are easily applied and their concentration can be controlled. These can be analyzed, purified if necessary, and the antimicrobial activity can be evaluated. Sunen [44] identified the minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic microorganisms associated with food. It was found that the effectiveness of inhibition varied with the type of commercial liquid smoke. Synthetic smokes are nearer to actual smoke curing, and harmful components can be eliminated from synthetic smokes.

The odor, composition of flavor compounds, and antimicrobial activity of the smoke are recognized to be highly dependent on the nature of the wood. Some studies have recognized beech and oak woods to produce wood smoke with the best sensory properties [45]. Further, herbs, spices (bay leaves, black peppers, cloves, coriander seed), or pinecones may also be added to produce unique aromatic smoke flavors [14]. Bacteriocin treatment was found effective inhibiting *Listeria monocytogenes* on salmon packaged under vacuum or modified atmosphere [46]. Lingbeck et al. [47] reviewed the functionality of liquid smoke as a natural antimicrobial in food preservation. The review showed that several common foodborne pathogens such as *Listeria monocytogenes*, *Salmonella*, pathogenic *Escherichia coli*, and *Staphylococcus* are sensitive to liquid smoke in food systems.

29.4 CONCLUSION

The main purpose of smoking is to provide desired sensory flavors and colors to the foods as well as to inactivate enzymes and to deliver an antimicrobial effect. Smoked products are commonly stored under chilled or refrigerated conditions, but they are high-risk products as compared to dried and frozen food products. In many instances, other preservation hurdles, such as herbs, spices, and nisin are used to enhance preservation and stability. The smoking processes are cold smoking, hot smoking, and liquid smoking, and each one has specific advantages and disadvantages. Quality management tools, such as GMP, HACCP (Hazard Analysis and Critical Control Point), and hygienic conditions are important to deliver high-quality and safe smoked foods.

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30 Osmotic Dehydration of Foods

Mohammad Shafiur Rahman

CONTENTS

30.1 THE OSMOTIC PROCESS AND ITS DYNAMICS

30.1.1 Osmotic Dehydration

Osmotic dehydration of foods has potential advantages in fruit and vegetable processing industries. This dehydration process generally does not produce a product of low moisture content to be considered shelf-stable. Consequently, the osmotically treated product should be further processed (generally by air-, freeze-, or vacuum-drying methods) to obtain a shelf-stable product or the process could be used as a pretreatment for canning, freezing, and minimal processing.

Osmotic dehydration is the process of water removal by immersion of cellular solid in a concentrated aqueous solution. The driving force for water removal is the concentration gradient between the solution and the intracellular fluid. If the membrane is perfectly semipermeable, solute is unable to diffuse through the membrane into the cells. However, it is difficult to obtain a perfect semipermeable membrane in food systems due to their complex internal structure, and there is always some solute diffusion into the food and leaching out of the food's own solute. Thus, mass transport in osmotic dehydration is actually a combination of simultaneous water and solute transfer processes (Figure 30.1).

The removal of water during the osmotic process is mainly by diffusion and capillary flow, whereas solute uptake or leaching is only by diffusion. The fundamental knowledge for prediction of mass transport is still a gray area, although considerable efforts have been made over the past decade to improve the understanding of mass transfer in osmotic dehydration [1]. Empirical models have been developed for mass transfer that are easy to use when kinetics parameters are correlated with dimensionless processing conditions [2]. Rastogi et al. [3] conducted a review on enhanced mass transfer.

The osmotic dehydration process can be characterized by equilibrium and dynamic periods [4]. In the dynamic period, the mass transfer rates are increased or decreased until equilibrium is reached. Equilibrium is the endpoint of the osmotic process, i.e., the net rate of mass transport is zero. Rahman

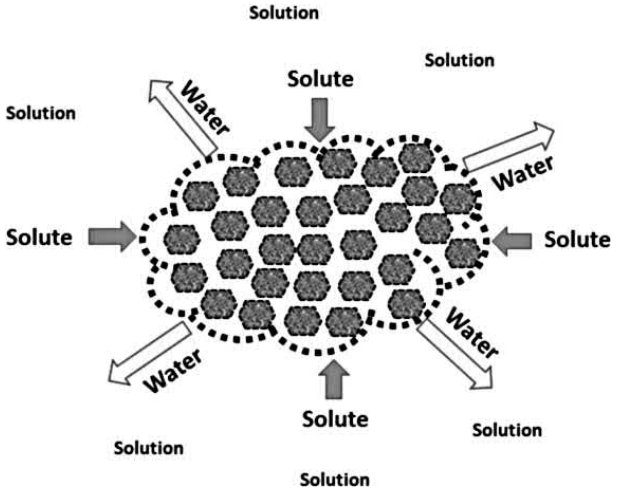

FIGURE 30.1 Water and solutes transfer in osmotic process.

[4] proposed equilibrium distribution coefficients for the *i*th component as

$$\lambda_i^e = \frac{X_i^e}{Y_i^o} \tag{30.1}$$

where λ_i^e is the distribution coefficient, and Y_i^o and X_i^e are the mass fractions (wet basis) of the *i*th component in the initial osmotic syrup and food product at equilibrium, respectively. The distribution coefficients for water can be defined as:

$$\lambda_w^e = \frac{X_w^e}{Y_w^o} \tag{30.2}$$

Similarly, the distribution coefficient for total solids can be defined as

$$\lambda_s^e = \frac{X_s^e}{Y_s^o} \tag{30.3}$$

The equilibrium coefficients varied with concentration, temperature, types of solutes, geometry, and food–syrup mass ratio. The $_w^e$ varied from 0.74 to 1.43 and $_s^e$ varied from 0.82 to 1.12 in case of osmotic dehydration of pineapple when temperature and syrup concentration varied from 30°C to 60°C and 50% to 75% (w/w), respectively [5]. Similarly, distribution coefficients were measured for pineapple [6], potato [7], apple [8], and mango [9]. In the case of apple, at constant syrup concentration, the distribution coefficient of water decreased and the distribution coefficient of solids increased with the increasing temperature. The influence of syrup concentration on distribution coefficients showed opposite trends, i.e., increasing syrup concentration increased the distribution coefficient of water but decreased the distribution coefficient of solids [8]. Sablani et al. [8] also developed correlations as a dimensionless function of temperature and syrup concentration. Sablani and Rahman [9] developed correlations for mango as

$$\lambda_w^e = 2.05\left(\frac{T}{T_r}\right)^{-1.60}\left(Y_s^o\right)^{0.397}\left(\frac{A_s}{l^2}\right)^{-0.007} \tag{30.4}$$

$$\lambda_s^e = 0.468\left(\frac{T}{T_r}\right)^{2.04}\left(Y_s^o\right)^{0.317}\left(\frac{A_s}{l^2}\right)^{0.023} \tag{30.5}$$

where T is the process temperature (K), T_r is the reference temperature (273 K), A_s is surface area of the sample (m^2), and l is the smallest geometric dimension of the sample (m).

30.1.2 Curing by Salts

Curing was originally developed to preserve certain foods by addition of sodium chloride. In the food industry, the curing is related only to certain meat, fish, and cheese products. Today sodium chloride, sodium, and potassium nitrite or nitrate are considered curing salts. Salting is one of the most common pretreatments used for fish products. Salting converts fresh fish into shelf-stable products by reducing the moisture content and acting as a preservation method. In combination with drying, these processes contribute to the development of characteristic sensory qualities in the products, which influence their utilization as food [10, 11]. Although curing was originally a mechanism for preservation by salting, over several millennia additional processes concomitant with curing have evolved, notably fermentation, smoking, drying, and heating. Curing may have different connotations: in meat, salt and nitrite or nitrate are always added; in fish, salt is always added, but nitrite only rarely; and in cheese, which always contains salt but infrequently contains nitrate. The term curing is applied to the production of desirable proteolytic and lipolytic changes. In the past half-century, cured products have been developed that are not stable unless refrigerated. Indeed, most cured meat products must be refrigerated to remain safe and wholesome, and even the packaging of many classes of cured products has become important in extending the shelf life [12]. Cured meats can be divided broadly into three groups: unheated, mildly heated (pasteurized to center temperature of 65–75°C), and severely heated (shelf-stable after heating to 100–120°C) [12].

In addition to the curing salts and related processes mentioned, additives collectively known as adjuncts are used in many cured meat products. These include ascorbates, phosphates, glucono-D-lactone, and sugars. Adjuncts are used primarily to obtain or maintain desirable changes; the ascorbates in connection with color; and the others in connection with pH, texture, and in some cases flavor. Adjuncts may also affect safety. The concentration of each curing agent depends on the nature of the food products and on the technology used in individual countries [12].

Salting can be done by placing fish in salt solution or covering with dry salt. During salting, water is being removed from the flesh, salt enters the tissues of the fish, and the body juices become a concentrated salt solution. When enough salt enters, it interacts with all the proteins causing coagulation. When the tissue cells shrink because of the loss of a large share of the moisture content, the fish flesh loses most of its translucent appearance and does not feel sticky to the touch. At this stage, it is said to be *struck through* [13]. A review of

the osmotic treatment of fish and meat products is provided by Collignan et al. [14].

30.2 EFFECTS OF OSMOSIS ON BIOLOGICAL MATERIALS

The cell viability of osmotic-treated apple revealed that the first layer of cells at a depth 1–2 mm from the surface died as a result of severe osmotic shock. The depth of the layer of severely injured or dead cells coincided with the penetration depth of the osmotic solute [15]. Cell viability of onion protoplast did not suffer any significant change regarding the nature of the sugar and the sucrose concentration. Sucrose solution dramatically decreased the cell viability in strawberry tissue [16]. During osmotic treatment, a redistribution of the components in cell membrane took place causing a reduction of the surface membrane available for swelling. It might explain that protoplasts swell until plasma membrane broke during the rehydration with isotonic solution with respect to fresh tissue. Maltose and trehalose had a protective effect on plasma membrane of onion epidermis cell, maintaining its properties as a barrier. Trehalose played a major role during the rehydration of onion epidermis, leading to the highest swelling rate. Contrarily, parenchymatic cells of strawberry tissue were not susceptible to any protective effect regarding the kind of disaccharides employed [16].

Electronic microscopy of strawberry [16, 17], apple [18], onion [16], orange peel [19], kiwifruit [20], and eggplant [19] was studied to explain the modification of cellular structure and disruption of cell membrane by the osmotic process. The rehydration kinetics of osmotic-pretreated and air-dried carrot slices decreased with the increased syrup concentration used in osmotic dehydration. In addition, solute loss increased during rehydration possibly due to structural changes induced by the osmotic process [21].

Water sorption characteristics of osmotically treated apple slices shifted to the right [22]. Osmotic treatment changed the moisture isotherm of air-dried sweet potato [23]. Salting or solute addition affects the air-drying process by reducing water diffusivity [24–27]. The concentration of the osmotic solution used in the pretreatment influenced the drying process, and a variable diffusivity and shrinkage were more suitable to describe the experimental data [28]. In the case of sweet potato, osmotic treatment with corn syrup did not affect the water diffusivity during air-drying [23]. Water diffusivity in strawberry during air-drying increased in the case of blanching or blanching followed by osmotic treatment [17]. The effective diffusivity of pomegranate arils during air-drying of ultrasound-assisted osmotic pretreatment was increased by 43% during the first falling rate period and by 66% during the second rate falling rate period. It was due to the structural changes for arils and the frequency of 40 kHz being more effective than 25 kHz [29]. Ultrasound during osmotic drying showed satisfactory results in terms of color, but increased hardness and decreased anthocyanin in air-dried arils. The ultrasound-assisted osmotic dehydration prior to hot air drying (60°C and air velocity 1.5 m/s) decreased the total drying time of persimmon fruit [30]. First, osmotic dehydration (30°C, 45°Brix) was performed at 35 kHz (10–30 min) followed by dehydration in 70°Brix sucrose solutions with shaking at 100 rpm. The air-drying rate increased 21% in the effective water diffusivity by 30 min. The pretreatment affected the rehydration rate and total phenolic content, while there was no significant difference between the color parameters.

Quality attributes and structure of hot air (1080 min drying and energy consumption 135.2 kJ/g), vacuum microwave (34 min drying, energy consumption 26.5 kJ/g), osmotic microwave (3 min drying, energy consumption 11.5 kJ/g), and osmotic vacuum microwave (11 min drying, energy consumption 24.0 kJ/g) drying of tilapia fillets were assessed [31]. Osmotic treatment was performed by submerging fish in a salt solution (20%, w/w) at 30°C for 2 h. The final moisture contents of all dried products were around 11.0 g/100 g sample. Vacuum drying promoted the formation of a porous structure. The shrinkage rate, rehydration rate, hardness, and elasticity of osmotic-vacuum-microwave fillets and vacuum-microwave fillets showed similar results. The osmotic vacuum microwave drying improved the quality of the fillets better in terms of microstructure, energy consumption, and color change. Therefore, combined methods could be better options for the quality of the dried products. The salt concentration has also great influence on the rate of surface evaporation [32]. In addition, depending on the salt concentration and relative humidity, the salted fish may reabsorb moisture from the environment during storage [33].

30.3 POTENTIAL ADVANTAGES FOR INDUSTRY

The use of the osmotic dehydration process in the food industry has several advantages: (i) quality improvement in terms of color, flavor, and texture; (ii) energy efficiency; (iii) packaging and distribution cost reduction; (iv) chemical treatment not required; and (v) product stability and retention of nutrients during storage.

30.3.1 Quality Improvement

It is well established that osmotic dehydration improves product quality in terms of color, flavor, pigment, vitamins, and texture. The merits of osmotic dehydration for product quality improvement and process efficiency were reviewed [1, 4, 34, 35]. Air-dried cashew apple pretreated with an osmotic solution of sodium chloride and sucrose showed improved color, firmer texture, astringency, and overall acceptability [36]. Fluidized bed air-dried blueberries pretreated with an osmotic solution showed low shrinkage, better rehydration, and soft raisin-like texture [37]. Agreeable flavor, color, and texture of osmo-air-dried peppers with whey and sorbitol were developed without preservatives [38]. Osmo-air-dried peas were developed with attractive color and acceptable organoleptic properties with preosmotic dehydration in sucrose and citrate solution [39]. Microwave-dried apple and strawberry pretreated with osmotic dehydration showed high quality in terms of color, taste, vitamin C, and structure retention.

Scanning electron microscopy (SEM) analysis revealed that the cellular structure was preserved better with osmotic treatment [40]. Structural and compositional profiles in osmotically dehydrated apple were studied by Cryo-SEM analysis [41]. The firmness of osmo-dehydro-frozen apple measured by oscillation rheometry showed in the following order: no treatment > sorbitol = sucrose > corn syrup > blanched [18]. Vacuum pulse did not favor either preservation of kiwi and mango mechanical response or their cryo-preservation. The mechanical response of strawberry was better preserved by air-drying osmotic treatment, but the freezing–thawing process provided similar results. Thus, it is necessary to optimize the process treatment based on each fruit [42]. Atmospheric-osmotic-treated kiwifruit with glucose, glycerol, or sucrose showed lower failure forces than fresh fruit. Calcium lactate infiltration or vacuum infusion increased failure forces due to enhanced cell cohesion and increased cell wall integrity. Relaxation tests showed that infusion (atmospheric or vacuum) sharply decreased the elastic component of rheological behavior of kiwifruit [20]. In the case of potato, longitudinal stiffness was affected much more by osmotic treatment than was shear stiffness [43].

In the literature, there is not much fundamental understanding of the mechanisms of flavor entrapment in the food matrix, color retention, and the physics of textural improvement. The phenomena that retain aroma are (i) adsorption of volatiles onto the infused solute matrix, (ii) physicochemical interactions between volatiles and other substances, and (iii) microregional encapsulation in which volatile compounds are immobilized in *cages* formed with the association of dissolved solids [44–50]. For papaya, the loss of hydrosoluble solutes, such as vitamin C could be reduced, most likely because of a sugar barrier layer that is formed at the periphery [51]. In case of kiwifruit, high temperatures and high sugar concentrations could reduce the loss of vitamin C and chlorophyll due to the preferential increase of water loss compared to solutes [52].

Processing at 50°C or more leads to disadvantageous modifications of color, ascorbic acid, and chlorophyll contents. Processing at 40°C or lower gave satisfactory ascorbic acid and pigment contents in the finished product. Furthermore, additions of ascorbic acid in syrup, as well as calcium and/or copper chlorides, can enrich the quality, in particular the color [52]. Talens et al. [53] found that osmotic treatment caused changes in the volatile profile of the kiwifruit depending on the treatment conditions. The concentration of the ester fraction increased, whereas aldehydes and alcohols decreased. Vacuum pulse application and process time promoted ester formation. The decrease in the aldehydes and alcohols was greater in treatments carried out at atmospheric pressure. After one month in frozen storage, a severe reduction of all compounds (esters, aldehydes, and alcohols) resulted in the same volatile profile in a treated and directly frozen sample. The use of high sucrose concentration (40–65%) in osmotic dehydration of kiwifruits can be recommended in order to minimize microbial adhesion and to slow microbial growth during storage, even if it has to be taken into account that high sugar concentration could determine problems of solubility and sugar recrystallization [54].

Minerals or functional substances could be fortified in vegetables and fruits by applying the osmotic process. The effect of Ca and Fe ions incorporation in the structural matrix of apple during the vacuum osmotic dehydration process at 30°C was studied by Barrera et al. [55]. Only calcium had the ability to strengthen the cell structure, which diminished effective diffusivities. Osmotic-dried apple showed much lower and uniform shrinkage compared to air-dried apple at the same level of moisture content [56]. Calcium infiltration in osmotic-treated apple showed better retention of cellular microstructure during subsequent high temperature and a short blanching process [57].

Osmotic pretreatment kept residues of the solute inside the product, influencing the taste and flavor of the product, as well as the dielectric properties. The increased salt concentration has a strong effect on the loss factor. Osmotic-treated mushrooms dried by microwave hot air produced more homogeneous heating due to the reduced center heating, slightly shorter drying time, improved rehydration properties, reduced shrinkage, and a higher open-pore porosity [58]. Fito et al. [19] explored the possibility of formulating functional, stable, and fresh-like products by incorporating minerals, vitamins, and health-beneficial functional components in the osmotic solution, in addition to water activity or pH depressors, and antimicrobial components.

Osmotic pretreatments (sugar, sodium chloride, and maltodextrin) affect the quality attributes of french fries [59]. Osmotic pretreatment decreases oil and moisture content of french fries, while the porosity increased and provided improved structure. Color darkening took place during osmotic dehydration, and browning reactions during frying are promoted resulting in a darker and red-colored fried product. Salt-treated samples have the most acceptable color.

Shrinkage and deformation of apple cubes of 1.5 cm showed significant deformation including cell and pore collapse during hot air drying at 80°C [60]. Only osmotic pretreatment or instant control pressure drop (DIC) could not improve the appearance and texture properties of air-dried apple cubes. However, osmotic pretreatment and DIC combined with maltodextrin or microcrystalline cellulose could increase crispness (i.e., 57–64; air-dried only: 10) and reduce hardness (i.e., 138–145 N; air-dried only: 15 N) of apple cubes. In addition, and the deformation of volume was increased by 1.6 times as compared with hot air drying alone. Therefore, combined technology could be used to obtain air-dried products with good appearance and texture properties.

30.3.2 Energy Efficiency

Osmotic dehydration is a less energy-intensive process than air- or vacuum-drying because it can be conducted at low temperatures. Energy consumption in osmotic dehydration at 40°C (with syrup reconcentration by evaporation) was at least two times lower than convection air-drying at 70°C [61]. In the frozen food industry, high energy levels are used

for freezing, because a large quantity of water is present in fresh foods. A significant proportion of this energy could be saved if plant materials were concentrated prior to freezing [62, 63]. A reduction in the moisture content of food can reduce refrigeration load during freezing. This is typified by salting, which is one of the oldest methods for preserving fish and vegetables. The high level of solute in osmotically treated products decreases water activity and preserves them, and thus energy-intensive drying processes can be avoided. Following osmotic treatment, the resultant syrup can be used in the juice or beverage industries as a by-product, thereby improving process economy.

Osmosis pretreatment with 7% (w/w) sodium chloride for 30–90 min showed no significant effect on energy and exergy loss of microwave air-drying (90–900 W) of 4 cm thick orange slices [64]. Increasing the microwave power increased the energy and exergy efficiency of drying, leading to a reduced drying time. Osmosis pretreatment increased the absorption of heat in orange, leading to an increase in the energy and exergy efficiency during air-drying. Osmosis time showed more effect on the energy efficiency than the exergy efficiency. Considering the effective moisture diffusivity and mass transfer coefficient, the optimum energy and exergy efficiency was considered at power 900 W and osmotic time 90 min. The maximum exergy loss was observed at 360 W and osmosis time of 60 min.

30.3.3 Packaging and Distribution Cost Reduction

Partially concentrating fruits and vegetables prior to freezing saves packaging and distribution costs [65]. The product quality is comparable with that of conventional products. The process is referred to as *dehydro-freezing.*

30.3.4 Reduction of Chemical Treatment

Canning of apples is not practiced commercially due to some inherent problems associated with the high gas volume in apple tissue and difficulty of its removal during exhausting, less drained weight, and mushy texture [66]. There have been a few attempts of canning apple slices by the use of calcium chloride, a firming agent, in order to improve texture [67]. However, using osmotic-treated apple pieces in the canning process resulted in a firmer texture and improved quality of the product [66]. This process is known as *osmo-canning.* Similarly, firmer apple slices were possible when calcium ion was used in the osmotic syrup [55].

Chemical treatment to reduce enzymatic browning can be avoided by the osmotic process [68]. There are two effects of sugar in producing a high-quality product: (i) effective inhibition of polyphenol oxidase, the enzyme that catalyzes oxidative browning of many cut fruits; and (ii) prevention of the loss of volatile flavor during further air- or vacuum-drying [69]. However, if the final product after air-drying contains 10–20% moisture, enzymatic and nonenzymatic browning cause slow deterioration of color and flavor [70]. Therefore, Ponting [70] suggested adding a blanching step after the osmotic process and using sulfur dioxide during or after the osmotic step if the final moisture content of the fruits and vegetables is more than 20%.

30.3.5 Product Stability during Storage

The product obtained by osmotic processing is more stable than untreated fruits and vegetables during storage due to low water activity by solute gain and water loss. At low water activity, deteriorative chemical reactions and growth and toxin production by microorganisms in the food are low. In the case of canning, using high-moisture fresh fruits and vegetables, water can flow from the product to the syrup brine causing dilution. This can be avoided using the osmo-canning to improve product stability [66]. Similarly, the use of osmo-dehydro-frozen apricot and peach cubes in yogurt can improve consistency and reduce whey separation of the yogurt [71]. Solute exerts a germicidal effect. Salt reduces the solubility of oxygen in the substrate, thereby, restricting the growth of aerobes.

30.4 FACTORS AFFECTING OSMOTIC DEHYDRATION PROCESS

The mass transport during osmotic dehydration depends on the factors described in the following sections.

30.4.1 Type of Osmotic Agent

The most commonly used osmotic agents are sucrose for fruit and sodium chloride for vegetables, fish, and meat. Other osmotic agents include glucose, fructose, lactose, dextrose, maltose, polysaccharide, maltodextrin, corn starch syrup, whey, sorbitol, ascorbic acid, citric acid, calcium chloride, and combinations of these osmotic agents. A number of researchers investigated the use of binary mixtures of solutes with sucrose as a means of reducing solute cost and improving the effectiveness of osmosis [72, 73]. The ultimate choice of blend will depend on many factors, such as solute cost, organoleptic compatibility with the end product, and additional preservative action by the solute. Calcium from a calcium chloride brine approximately iso-osmotic with cucumber cell sap penetrated cucumber fruit much more slowly than did calcium ions from a mixture of sodium chloride and calcium chloride. After 96 h, calcium that penetrated from the isoosmotic brine was localized mostly in the exocarp and relatively little in the interior tissues, while calcium that penetrated from the supa-osmotic brine reached higher concentrations and was more evenly distributed among the exocarp, mesocarp, and endocarp sections [74]. It was A synergistic action of two solutes (sucrose and sodium chloride) was observed on soluble solids gain in apple. With respect to the sensory analysis of apple samples dehydrated in ternary solutions, it is possible to assert that salt gain was not sufficient to balance the excessive sweetness of the product processed at high sucrose concentrations. Small additions of sodium chloride (up to 1%) did not determine the complete decay of product acceptability if coupled with limited sucrose concentration (<54.5%) [75].

30.4.2 Concentration of Osmotic Solution

Both the water loss to equilibrium level and osmotic drying rate increase with the increase in osmotic syrup concentration, since water activity (i.e., mass transfer driving force) of syrup decreases with the increase in solute concentration in the syrup [65, 76–82]. A dense solute-barrier layer at the surface of the product is formed with increase in syrup concentration, thus enhancing the dewatering effect and reducing the loss of nutrients during the process [83, 84]. A similar solute barrier is also formed in the case of syrups with higher molecular weight solutes at even low concentration.

30.4.3 Temperature of Osmotic Solution

The rate of osmosis is markedly affected by temperature [85]. This is the most important parameter affecting the kinetics of water loss and solute gain. Water loss increases with increase of temperature, whereas solid gain is less affected by temperature. In the case of high temperature, solute cannot diffuse as easily as water through the cell membrane and thus the approach to osmotic equilibrium is achieved primarily by the flow of water from the cell [82]. This type of equilibrium results in a lower solute gain by the product. Askar [86] recommended a high temperature and short time for osmo-solar-dried peach since it can produce good texture with high aroma and color retention. Main influence in osmotic dehydration was observed at a high temperature of 50°C, and at 26°C no appreciable change in solids concentration was observed at distances deeper than 0.5 cm from the cube surfaces even at 168 h. At 50°C, all the layers were affected even at shorter times (8 h) [87].

Fish are salted over the temperature range of 0°C to 38°C. The higher the temperature, the faster the salt infusion and the quicker the process reaches equilibrium. In general, fish absorbs salt faster as the brining temperature increases. It is best to standardize brining at a cool temperature (1.1–1.7°C) to achieve consistent and predictable results and to discourage bacteria growth. Using ice in the brine can accomplish this, but caution must be used to make sure no ice remains in the finished brine. Brining in a cold room is also a good way to keep brines cool and is advisable for long brining times [88].

In general, salt absorption is affected by brine concentration and temperature, brining time, thickness and geometry of fish, texture and fat content of fish, species, and fish quality [88]. Fish flesh absorbs salt faster from higher salt brine concentration. Brine greater than 15.8% tends to remove moisture from the fish, which can be advantageous in some products. However, strong brines and short times may not allow even distribution of salt into the center of the fish geometry prior to smoking. Dry salting has the advantage of removing moisture, but has the disadvantage of uneven salt absorption. Dry salting is a technique that covers fish with a thin layer of salt (0.64–1.27 cm) between layers of fish [88]. Tilapia was processed by dry salting (fish:salt ratio, 3:1) varying salting time (0–24 h), air-drying time (6–20 h), and drying temperature (40–60°C). The critical salting times for attaining minimum moisture were 20.5, 12 and 8.5 h, respectively, for products air-dried at 40°C, 50°C, and 60°C. The hardness, color, and overall acceptability of salted dried tilapia were found to be dependent on the process variables, salting time, drying time, and temperature [33].

30.4.4 Properties of Solute Used in Osmosis

The osmotic process is affected by the physicochemical properties of the solutes employed. These differences arise mainly from differences in molecular weight, ionic state, and solubility of the solute in water. According to the principle of osmosis, the rate of water loss from the fruit to the syrup having large molecular weight solute is lower than that of syrup having small molecular weight solutes when both syrups are at the same mass concentration. This is due to low vapor pressure of the syrup having low molecular weight solute. However, contrary to this physical chemistry principle, for osmotic syrups with equal concentrations at the early stage, those with high molecular weight solutes will have a greater rate of water removal and lower solute transfer (due to low solute penetration) than those of low molecular weight. This was demonstrated by a model agar gel [89] and apple [90].

The pH of the syrup can also affect the osmotic process. Acidification increases the rate of water removal by changes in tissue properties and consequential changes in the texture of fruits and vegetables [91]. Water removal was maximal at pH 3 for apple rings using corn syrup [92]. In a more acidic solution (i.e., at pH 2), the apple ring became very soft. However, firmness was maintained at pH values within 3 to 6. The softening may be due to hydrolysis and depolymerization of the pectin.

30.4.5 Agitation of Osmotic Solution

Osmotic dehydration can be enhanced by agitation or circulation of the syrup around the sample [72, 78, 92]. However, the improvement is so small that in some cases it might be more economical to use no agitation when consideration is taken of equipment needs and breaking of fruits [68]. The effect of agitation on the osmotic dehydration of kiwifruit slices was found to depend on the ratio of syrup to fruit mass and syrup concentration [93].

30.4.6 Material Geometry

Osmotic concentration behavior depends on the geometry of sample pieces, due to the variation of surface area per unit volume (or mass) and diffusion length of water and solutes involved in mass transport. Contreras and Smyrl [92] found that mass loss was about 1.3 times higher when apple slice thickness decreased from 10 mm to 5 mm. Lerici et al. [79] found that solute gain increased as the ratio of surface area to minimum diffusion length increased, while water loss increased to a maximum (depending on the shape) and then decreased. This decrease in water loss was probably due to a reduction of diffusion caused by high solid gain at the surface

and consequent formation of a solute layer. At the same operating conditions, fresh fruit having different sizes and shapes can give final products with very different characteristics [79]. In the case of osmotic dehydration of apple, water loss and solids gain generally increased with contact time, temperature, and concentration, and decreased with an increase in size of cylindrical sample [94].

30.4.7 Osmotic Solution and Food–Mass Ratio

Both solid gain and water loss increase with the increase of the ratio of syrup to food mass [68, 95]. Uddin and Islam [96] studied the effect of the ratio of syrup and fruit slice mass on the osmotic treatment of pineapple at 21°C. They observed that weight loss increased until the syrup-to-fruit ratio was 4:1, but by increasing the ratio up to 6:1 no further gain was observed. Thus, they defined the optimum ratio as 4:1 for pineapple. The rate of solid gain and water loss increased significantly: the ratio of syrup to fruit mass was increased from 1:1 to 6:1 in case of osmotic drying of potato in 61% sucrose syrup [61]. At equilibrium, the solute content in potato was the same at syrup–fruit ratios of 1:1 and 10:1. Thus, the ratio of syrup to fruit mass has a negligible effect on potato composition at equilibrium.

30.4.8 Physicochemical Properties of Food Materials

The chemical composition (protein, carbohydrate, fat, salt, etc.), physical structure (porosity, arrangement of the cells, fiber orientation, and skin) and pretreatments may affect the kinetics of osmosis in food. A steam-blanching step for 4 min before osmosis gave lower water loss and higher solid gain when applied to fresh potato slices [73]. The loss of membrane integrity due to heating was the cause of the poor osmotic concentration behavior [73]. Freezing raw fruit disrupts cells and results in poor osmosis of thawed fruit [70]. Saurel et al. [84] studied osmosis of frozen apple without thawing in ethylene glycol and polyethylene glycol at 30°C to 70°C. They observed similar results as fresh apple.

Soft-textured fish tend to absorb salt faster than tough or firm-textured fish. Frozen flesh absorbs negligible salt, and thus need thawing. Mishandled fish with gaping (separated flesh fibers) may have decreased brining times. High-fat-content fish absorb salt slower than low-fat fish. However, they may need less salt to obtain adequate final water phase salt content. Fat content in flesh varies at different locations on the body of the fish. Salmon, for example, tend to have less fat at the tail. Different species of fish have different flesh characteristics and may absorb salt at different rates. Salting times should be specific for each species. Moreover, geometrical shapes of fish having different thickness and width along the length also make it difficult to control the salting process and causes nonuniform salt distribution. Frozen-thawed fish or low-quality fish have flesh characteristics that may affect (usually increase) the rate of salt absorption. The rate of freezing affects flesh cell structure and therefore the subsequent rate of salt absorption [88]. In some cases, for example in the case of salmon, the fish is soaked overnight in fresh water or for a period of 12–16 h before curing. The water is changed two or three times. Ten or 12 h of freshening should be sufficient, but a more thorough soaking may be required to satisfy some markets.

Diffusivity of manganese ion in cured pork was varied $0.42–1.0 \times 10^{-10}$ m^2/s [97]. Salt diffusion in pork meat was found $3.6–1.2\ 10^{-10}$ m^2/s (temperature –2°C to 36°C) and for fat it was $0.07\ 10^{-10}$ m^2/s [98]. Salt has a profound effect on the ultrastructure and hence moisture binding of fish muscle. It has a greater effect compared to freezing, drying, or heat treatment [99].

30.4.9 Operating Pressure and Other Forces

Vacuum osmotic dehydration results in a change of behavior of mass transfer in fruit–sugar or salt–solution systems [93, 100–105]. Vacuum treatments intensify the capillary flow and increase water transfer, but have no influence on solute uptake [101]. The total water transfer results from a combination of traditional diffusion and capillary flow, and it is affected by the porosity or void fraction of the fruit [103, 105]. The reduction in pressure causes the expansion and escape of gas occluded in the pores, and pores can be occupied by osmotic solution, thus increasing the mass transfer rate. Pulsed vacuum osmotic drying proved effective for figs with higher water loss and solids gain [28].

In addition to vacuum drying, applications of high hydrostatic pressure [106], centrifugal force [107], high electric pulse [108, 109], and ultrasound [110] are used to improve the mass transfer [3]. The increased mass transfer in high electric field and high pressure was due to the irreversible increase in cell wall permeability [106], and the initiation and growth of the pores were time-dependent [108]. Petrotos and Lazarides [111] reviewed osmotic concentration of liquid foods by applying osmotic membrane techniques, namely, direct osmosis, membrane distillation, and osmotic distillation.

The acoustic increases the mass transfer by reducing the thickness of the boundary layer by generating localized pressure fluctuations [112]. Ultrasound (i.e., 25 and 40 kHz at different intervals) was applied to enhance the efficiency of moisture removal during the osmotic dehydration of pomegranate arils [29]. It was observed that more detachment of cell wall increased water loss (i.e., 2-fold and 2.7-fold increment for water loss at the frequencies of 25 and 40 kHz, respectively) and solute gain. Microscopic analysis of ultrasound-assisted (20 kHz, 130 W) osmotic dehydration (i.e. solutes: sodium chloride and 12 DE maltodextrin) showed the formation of microchannels due to the loss of cellular adhesion, which produced large cell interspaces and other tissue structures of potato [113]. This resulted in higher moisture and solid mass transfer. At solute concentration of 30%, ultrasound-assisted osmotic dehydration enhanced the effective diffusivity of water by about 5.5–260%, whereas the ultrasound pretreatment in water increased up to 130% when solute concentration of 70% was used in osmosis. A more intense dense cell breakdown was observed when ultrasounds were

applied in the osmotic process as compared to application before the osmotic dehydration process. The solids gain of potato cubes submitted to ultrasound pretreatment was lower than that of ultrasound-assisted osmosis, thus reduced calorie low sugar or salt products could be achieved.

Water loss of garlic slices treated with vacuum osmotic dehydration (21.12%) was higher than slices treated with normal osmotic dehydration (10.67%), while vacuum pretreatment osmotic and multifrequency mode ultrasound-assisted osmotic dehydration showed water loss as 14.1% and 11.2–13.6%, respectively [114]. Low-field nuclear magnetic resonance showed the moisture migration in the vacuole, cytoplasm and intercellular space, and the cell wall of garlic cells. The allicin content, surface color change and firmness of vacuum-osmotic treated garlic slices were better than the other treated samples.

30.5 CHALLENGES TO APPLICATION IN THE FOOD INDUSTRY

There are a number of technological problems to be overcome before the osmotic process can be applied to the food industry. These are discussed in the following sections.

30.5.1 Product Sensory Quality

The main disadvantage of the osmotic process is that it may increase the saltiness or sweetness, or decrease the acidity of the product, which may not be desired in some cases. This can be avoided by controlling the solute diffusion and optimizing the process to improve the sensory assessment of the product. Adequate protective edible coating enriched with specific antimicrobial additives can be used to reduce surface microbial growth and other barriers during storage [115]. Edible semipermeable membrane coatings can also be used to reduce solute uptake and increase water loss [116]. Salting of cod fillets was achieved in osmotic solutions (sucrose and salt, or corn starch syrup and salt) with high molecular solutes allowed better control of salt entrance, thus produced light-salted fish [117]. However, microbiological and organoleptic validations need to be carried out for complete process validation.

30.5.2 Syrup Management

Syrup management with the batch process is difficult due to the dilution of solution and microbial contamination (Figure 30.2). The microbial validation of the process for long-time operation and reuse of the syrup by recycling are important factors for industrial applications [1]. The management of syrup is the major challenge to make the process industrially viable. These include syrup composition and concentration, syrup recycling, solute addition, reuse of the syrup, and waste disposal. The cost of the syrup is a key factor for the success of the process. The compositional changes related to leaching from the fruit or vegetable may influence the product quality (such as color, acids, sugar, minerals, and vitamins).

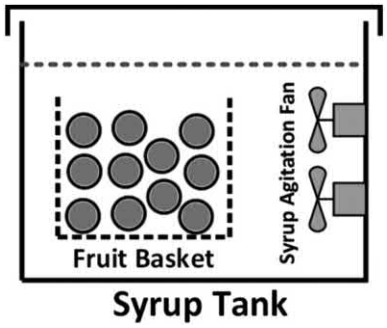

FIGURE 30.2 Schematic diagram of a batch osmotic process.

A continuous osmotic process is shown in Figure 30.3, where diluted solution is concentrated and recycled back to the osmotic process. Microbial contamination can increase with the number of times that the syrup is recycled. Depending on the environmental process conditions, the microbial load after several osmotic cycles can range from 2 10^2 cfu/ml to high levels of yeast and fungi only after 15th cycle, and 10^5 cfu/ml after 8 h of continuous treatment [118]. A pilot recycling system was validated for salt in the case of meat and fish. Over a 6-day processing period, the microbial load of the brine was kept at a low level (8.37 10^4 cfu/g of fungi; 1.19 10^3 cfu/g of yeasts and molds), and the mass transfer potential of the solution (water loss and salt gain) remained steady [14]. The options to reduce microbial contamination are more care for low-acid foods, avoid too much dilution of the solution, the processing environment should use an air-filtering system, and hygienic management of osmotic solution [118]. The control of solute composition in recycling for single-solute syrups is easier than mixed-solute syrups.

Barranco et al. [119] considered the following options for osmotic solution management: (i) direct reuse after some degree of regeneration; (ii) reuse with the final product, after preconditioning or a partial regeneration process; and (iii) use of the spent solution to produce substances of interest. These could be obtained either directly or through a microbiological process, and (iv) treated with a specific process. During the recycling process, the diluted syrup can be reconcentrated by evaporation, reverse osmosis, membrane filtration, freeze concentration, and/or treated with active carbon [14, 118, 119]. The unused syrup needs to be adequately treated before disposal in wastewater [118, 119]. The ratio of syrup to fruit mass

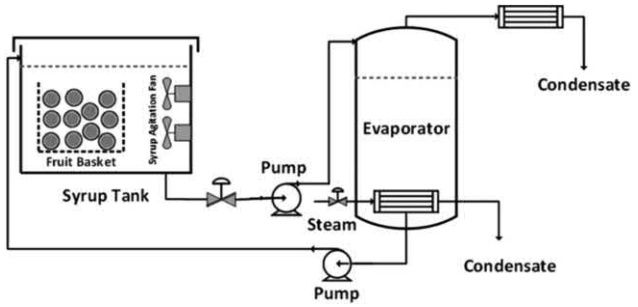

FIGURE 30.3 A process flow diagram of a continuous osmotic process.

should be kept as low as possible to reduce the production costs. Torreggiani [120] emphasized that the problems of the microbial growth in syrup and the effects of repeated reconcentration and recycling of syrup on its properties, need to be overcome in order to develop large-scale industrial process. Acidifying sucrose could result from the acidic fruits (or added) and this could increase the rate of osmosis; however, this caused darkening and quality losses during the subsequent drying process. Kubiak et al. [121] studied the effect of pH (4–10), temperature (50–60°C) and sucrose concentration (50–70°Brix) on sucrose hydrolysis rate. The sucrose hydrolysis at pH rage of 6–10 was insignificant and did not depend on temperature and sucrose concentration. Sucrose hydrolysis significantly increased and depended on hydrogen ion and sucrose concentration, temperature, and time with pH values below 5.0.

30.5.3 Process Control and Design

Inadequate information about the experiments presented in the literature and limited data available have precluded effective design and control of this process by the food industry. Further studies are necessary to get a clear understanding of the variation of equilibrium and rate constants with process variables and characteristics of the food materials. Most of the osmosis studies have been concerned with the qualitative prediction of the processing factors, but more quantitative prediction is necessary for industrial use in process design and control. On-line measurements of syrup properties can provide continuous control of the process. Fruits and vegetables tend to float on the concentrated syrup due to the higher density of the syrup. Moreover, the viscosity of the syrup exerts considerable mass transfer resistance causing difficulty in agitation, and the syrup tends to adhere to the surface of the food material. Breakage of fruit or vegetable pieces may occur by flow of syrup in the case of the continuous process or by mechanical agitation in the case of the batch process.

Marouze et al. [122] provided different possible equipment (batch and continuous processes) designs for osmotic dehydration. They found the following need to be considered:

(i) Creating relative movement between the solution and the food, characterized by relative speed and homogeneity for all food
(ii) Control of treatment time and for equipment used for continuous processing, and control of the spread of residence times in the contactor to ensure homogeneous treatment of the food
(iii) Ability to accept different shapes of food (whole or in cubes, slices, or fillets)
(iv) Reduction of the ratio of solution mass to food mass (a low mass ratio is of particular interest if the cost of the solution is high; it also restricts equipment size)
(v) Avoidance of oxidation in food in contact with air
(vi) Allowing a system for the solution to be introduced and removed
(vii) Allowing the food to be introduced and removed (for continuous processing: continuous introduction, and removal of food when it has reached required state of treatment)
(viii) Allowing control of the process control parameters (food and solution temperature, solution concentration, static pressure of food and solution, agitation)
(ix) Complying with the appropriate mechanical, electrical, and food-related standards
(x) Reasonable cost of equipment manufacture

Equilibrium is the endpoint of osmosis, but for practical purposes a number of other factors should be considered to ensure the quality of the final product. These include damage to the cells and development of off-flavor due to longer processing time as well as reuse of the syrup [4]. Finally, adequate packaging systems should be used to make sure that consumers are getting good sensory quality and safe products.

30.6 CONCLUSION

The osmotic process is widely used as a pretreatment for developing many shelf-stable dried, frozen, canned, and intermediate-moisture food products. The main advantage of this process is to improve the product quality attributes. The process dynamics depend on many factors, such as types and properties of osmotic agents; solution concentration, temperature, and agitation; the food's size, shape, and physicochemical properties; osmotic and food mass ratio; and other external applied forces. Other external forces, such vacuum and high pressure, centrifugal force, high electric pulse, and ultrasound are also used to enhance the mass transfer as well as to improve the quality. The main issue of the osmotic process is the inclusion of the solutes in the product, and syrup management, process design, and control are the challenges to be addressed.

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31 Fundamentals of Water Activity Concept

Mohammad Shafiur Rahman and Theodore P. Labuza

CONTENTS

31.1 IMPORTANCE OF WATER ACTIVITY

Water is an important constituent of all foods. Why water activity and not water content? In the middle of the 19th century, scientists began to discover the existence of a relationship between water in a food and its relative tendency to spoil. They also began to realize that the *active water* could be much more important to the stability (i.e., reactivity) of food than the total amount of water present. Scott [1] clearly identified that the water activity of a medium correlated well with the deterioration of food stability due to the growth of microorganisms. Thus, it is possible to develop generalized rules or limits for the stability of foods by using water activity. This was the main reason why food scientists started to emphasize water activity rather than total water content. Since then, the scientific community has explored the great significance of water activity in determining the physical, chemical, and sensory characteristics; effects of processing; and shelf-life prediction of foods.

Other applications of water activity extend to the process design and control, ingredients, and packaging selection. Water activity data are important to food processing, such as osmotic dehydration and air-drying. In drying operations, desorption isotherms at the process temperature are needed for design and control purposes. The endpoint of drying or the osmotic dehydration process could be determined from the equilibrium moisture content. In the drying process, the foods are equilibrated

to the air equilibrium relative humidity (i.e., water activity); in osmotic or salting processes, foods equilibrate with the osmotic solution water activity. Hence, water activity plays an important role in designing, operation, and control of drying processes and reverse osmosis. Water activity depressing power of solutes need to be considered when selecting ingredients or additives for food product formulation. When food materials are packed in a semipermeable membrane, foods could adsorb moisture if their water activity is lower than the external relative humidity of the air or could lose moisture if their water activity is higher than the relative humidity. The sorption isotherm is necessary to predict the moisture transfer rate through the packaging film and edible food coating, and so that shelf life can be predicted based on the water gained through packaging. The mathematical equations used to determine the isotherms for moisture transfer through packaging material are available in the literature [2, 3]. The water activity of fresh foods, as shown by Chirife and Fontan [4], is 0.970–0.996. It is important to understand the terminologies related to the water activity concept and factors affecting it in order to properly utilize it in food preservation and processing.

31.2 THERMODYNAMICS OF WATER ACTIVITY

Water activity, a thermodynamic property, is defined as the ratio of the vapor pressure of water in a system and the vapor pressure of pure water at the same temperature, or the equilibrium relative humidity of the air surrounding the system at the same temperature (Figure 31.1). Water activity is

$$a_w = \frac{\text{Vapor Pressure of Water in Food}}{\text{Vapor Pressure of Pure Water}} = \text{ERH} \quad (31.1)$$

where ERH is the equilibrium relative humidity of the air around the foods. A number of methods have been reported in the literature to measure or estimate the water activity of foods. Water activity measurement methods include the following: equilibrium sorption rate method (isopiestic method), vapor pressure measurement method, and hygrometric instrument method. In addition, water activity can be predicted from other thermodynamic properties, such as freezing point. The accuracy of most methods lies in the range of 0.01–0.02 water activity units [5]. Details of the various measurement techniques are described by Labuza et al. [6], Rizvi [5], Rahman [7], Rahman and Sablani [8], Rahman et al. [9], Fontana [10], Sablani et al. [11], and Rahman and Al-Belushi [12].

Water activity can be lowered or controlled by several methods, such as separating water (e.g., drying, baking), and/or adding solutes. Processes that can be used to remove water are drying, concentration, and dewatering by centrifuge. Solutes can be added to foods to reduce water activity as well as to improve the functional and sensory properties of foods, for example, adding salt to meat and fish and adding sugars to fruits. Specific antimicrobial effects and the cost of solutes or humectants should be considered for food product formulation when solutes are used to reduce the water activity. The factors affecting the selection of humectants are summarized in Table 31.1.

31.3 SORPTION ISOTHERM

The moisture sorption isotherm is the relationship of moisture content with the water activity of a food at a specified temperature. It is usually presented in graphical form or as an equation. Brunauer et al. [13] classified adsorption isotherms of materials into five general types (Figure 31.2). If water-soluble crystalline components are present in foods, e.g., sugars or salts, the isotherm appears as concave shape type III. Most other foods result in sigmoid isotherm type II. The inflection point of the isotherm indicates the change of water-binding capacity or of the relative amounts of free and bound water. Type I is indicative of a nonswelling porous solid, such as silicate (i.e., anticaking agent). For most practical purposes, the isotherm is presented in an empirical or theoretical model equation. However, none of the isotherm models in the literature are valid over the entire water activity scale of 0–1. The GAB model is one of the most widely accepted models for foods over a wide range of water activities from 0.10 to 0.9. The details of the isotherm models with their parameters are compiled by Rizvi [5], Okos et al. [14], Lomauro et al. [15], Lomauro et al. [16], and Rahman [7].

31.3.1 Hysteresis

The difference in the equilibrium moisture content between the adsorption and desorption curves is called hysteresis and

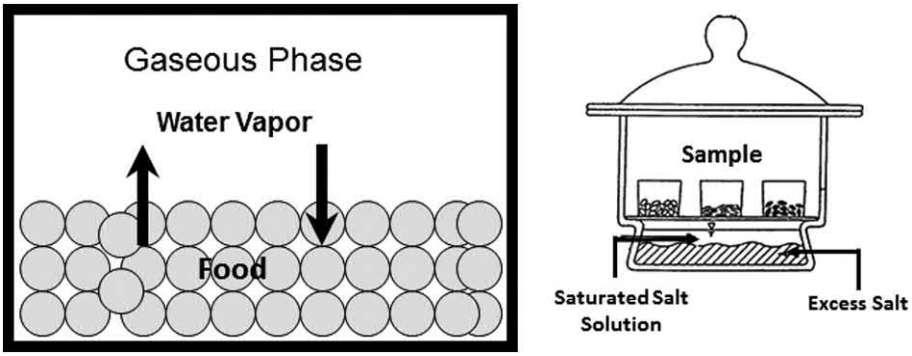

FIGURE 31.1 Thermodynamics of water transfer within food and the environment in a closed chamber.

TABLE 31.1
Some Criteria for Humectants to Be Used in Foods

Safe
Approved by regulatory agencies
Effective at reasonable concentrations
Compatible with the nature of food
Flavorless at used concentrations
Colorless and/or imparts no color changes in food

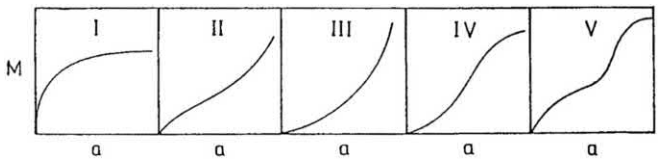

FIGURE 31.2 The five types of van der Waals adsorption isotherms proposed by Brunauer et al. [13].

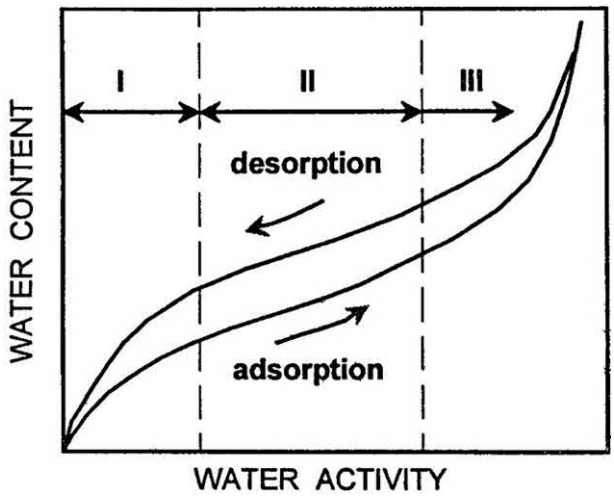

FIGURE 31.3 Sorption isotherm for typical food product showing the hysteresis.

shown in Figure 31.3. In region II of this figure, the water is less tightly held and is usually present in small capillaries; whereas in region III, water is held loosely in large capillaries or is free [17]. Hysteresis in sorption has important theoretical and practical implications in foods. The theoretical implications are evidence of the irreversible sorption process and the validity of the equations derived based on equilibrium thermodynamic functions. The practical implications deal with the effects of hysteresis on chemical and microbiological deterioration, and its importance on low- and intermediate-moisture foods [18]. The changes in hysteresis could be used as an index of quality deterioration, since hysteresis loops of foods change with storage time [19, 20], however this is not a widely accepted method of evaluation. Rahman and Al-Belushi [12] present a review on the sorption hysteresis in foods.

31.3.1.1 Factors Affecting Hysteresis

The desorption hysteresis loop usually ends at the monolayer, but in some cases, it extends down to an activity of zero [21]. In foods, a variety of hysteresis loop shapes can be observed, depending on the types of foods and the temperature [22]. The principal factors affecting hysteresis are composition of the product, isotherm temperature, storage time before isotherm measurement, pretreatments, drying temperature, and the number of successive adsorption and desorption cycles.

Variations in hysteresis can be grouped into three types of foods [18]:

(i) Hysteresis in high-sugar foods—In high-sugar or high-pectin foods, such as air-dried apple, hysteresis occurs mainly in the monomolecular layer water region, below the first inflection point of isotherm region I in Figure 31.3 [14]. Although the total hysteresis is large, there is no hysteresis above 0.65.
(ii) Hysteresis in high-protein foods—In pork, a moderate hysteresis begins at about 0.85 (i.e., in the capillary condensation region) [23].
(iii) Hysteresis in high-starch foods—In starchy foods, a large hysteresis loop occurs, with a maximum water activity of about 0.70, which is within the capillary condensation region [14].

Total hysteresis decreases as sorption temperature increases [22]. Desorption isotherms usually give a higher water content than adsorption isotherms. Chinachoti and Steinberg [24] found hysteresis in sugar containing starch up to 0.60 and Bolin [25] in resin (with very high sugar content) up to 0.30. Tsami et al. [26] observed significant hysteresis below 0.5 or 0.6 and at temperatures above 30°C in fruits (raisin, currant, fig, prune, and apricot). They mentioned that the absence of hysteresis at higher temperatures was due to the dissolution of sugars at high temperatures. The water activity below which a significant hysteresis effect was manifested was inversely proportional to the sugar content of the fruits [26]. In high sugar or high pectin foods such as air-dried apple, hysteresis occurs mainly in the monomolecular layer of water region [14]. Although the total hysteresis is large, there is no hysteresis above 0.65.

In the case of pork, a moderate hysteresis begins at about 0.85 (i.e., in the capillary condensation region) [23]. In starchy foods, a large hysteresis loop occurs with a maximum at about water activity 0.70, which is within the capillary condensation region [14], whereas in kudzu starch, hysteresis continues up to 0.90 [27]. Increasing temperature decreases the total hysteresis [20]. Iglesias and Chirife [28] estimated and compared the isosteric heat of water adsorption and desorption for a number of foods, and reported that the effect of temperature on the magnitude of hysteresis varied. There was no direct relationship between the observed differences in adsorption and desorption heats and the distribution of hysteresis along the isotherm. For some foods (thyme, winter savoy, sweet marjoram, cooked trout, raw and cooked chicken, and tapioca), increasing temperature decreased or eliminated hysteresis,

while for others the total hysteresis remained constant (ginger and nutmeg) or even increased (anise, cinnamon, chamomile, and coriander) [18].

The type of changes encountered upon adsorption and desorption depends on the (i) initial state of the sorbent (amorphous versus crystalline), (ii) transitions taking place during adsorption, the (iii) final water activity adsorption point, and (iv) sorption rate. If the saturation point has been reached and the material has gone into sorption, rapid desorption may preserve the amorphous state of supersaturated solution [18]. Some water remained after desorption (even after prolonged storage) due to hydrogen-bonded trapped water in amorphous sugar microregions as well as crystalline water [20, 29].

In some cases, hysteresis seems to be reproducible a second time [19, 30], and for some cases the second sorption–desorption cycle resulted in the elimination of hysteresis [31]. Elimination of hysteresis upon the second or subsequent cycles may take place for a variety of reasons, such as the change in crystalline structure when a new crystalline form persists in subsequent cycles [32], swelling and loss of elasticity (i.e. ability to return its original structure) of capillary walls resulting in a loss of ability to retain trapped water [33–35]. This loss of elasticity is due to the denaturation effects of surface-active agents [36], and mechanical treatments, and these may affect the capillary structure [18].

31.3.1.2 Theories of Sorption Hysteresis

Several theories have been formulated to explain the phenomenon of hysteresis, and at present a complete theory to encompass several responsible mechanisms is missing [26], and quantitative prediction of hysteresis is a challenge. Several theories have been proposed to explain hysteresis. The mechanisms proposed in the literature to cause hysteresis are discussed in the following sections.

31.3.1.2.1 Capillary Condensation

Capillary condensation can mostly account for hysteresis in nonswelling porous solids and can be explained using the Kelvin equation. Due to the presence of impurities, such as dissolved gas, the contact angle of the receding film upon desorption is smaller than that of the advancing film upon adsorption. Therefore, capillary condensation forms along the adsorbed moisture, and the sorption isotherm results in a higher relative vapor pressure [23].

31.3.1.2.2 Ink Bottle Theory

Rao [33] assumed capillaries to be composed of narrow necks with a large pore, somewhat like an ink bottle. On adsorption, the capillary does not completely fill until the water activity corresponding to the large radius of the pore is reached. During desorption, the smaller radius of the pore neck controls the emptying of the capillary, resulting in considerably lower water activity [21]. This theory was confirmed by Labuza and Rutman [37] for a cellulose model system. Cohan [38] elaborated upon the open-pore theory by extending the bottleneck theory, including considerations of multilayer adsorption. This was based on the difference as affected by the shape of the meniscus.

31.3.1.2.3 Mechanisms of Physicochemical Changes

The physicochemical changes in food components also cause hysteresis, such as deformability and elastic stresses of the sorbent, a deformation of the polypeptide chains within the protein molecule [18], and the energy surplus of unfolding (swollen) protein phase transition [39]. Kapsalis [18] discussed that adsorption from the dry state by biopolymers is due to (i) side-chain amino groups, (ii) end carboxylic and other groups, (iii) peptide bonds, and (iv) secondary structure.

In general, below 0.5 water activity, the main sites of sorption are the polar side-chain groups. The contribution of the polypeptide chain becomes progressively more important at higher activities, for example, at 0.80 activity the peptide bonds account for almost half the adsorbed water in wool keratin. Deamination of methylation of side-chain groups in wool and benzoylation in casein did not show any appreciable changes of hysteresis [40]. This suggested that it was the main chains of the biopolymers that are primarily responsible [18]. Sheehof et al. [41] supported the polar group interpretation of hysteresis, where binding mainly involves the free basic groups of the protein.

Kapsalis [18] showed a correlation of the maximum amount of hysteresis with the sum of arginine, histidine, lysine, and cystine groupings. Besides the free basic groups of the protein molecule, sulfur linkages are also of prime importance in hysteresis [42]. In contrast to this work, hysteresis in casein was observed to be independent of the free amino groups [40]. Thus, a two-fold nature of hysteresis was proposed: constant hysteresis, independent of the relative humidity desorption point; and hysteresis proportional to the amount adsorbed above the upper adsorption break of the isotherm 18]. In a swelling polymer, hysteresis depends on the mechanical constants contributed by the elastic properties and cannot be interpreted by capillary condensation [43]. Van Olphen [44] described retardation of adsorption due to the development of elastic stress in crystallites during the initial peripheral penetration of water between the unit layers. The shift toward higher relative vapor pressure during adsorption is caused by the required activation energy to open the unit layer stacks. The glass–rubber transition during adsorption and desorption also causes hysteresis due to the nonequilibrium state of the phase transition.

31.3.1.2.4 Structural Collapse

With sorption, the capillary pores of the adsorbent become elastic and swell. Upon desorption, the removal of water causes shrinkage, and a general collapse of the capillary porous structure occurs. Alteration of structure causes the elimination of hysteresis due to the absence of capillary condensation [18]. The collapse of capillaries during desorption also affects sorption hysteresis.

31.3.2 Water Activity Shift in the Isotherm

Typical water activity shifts by temperature at constant moisture content are shown in Figure 31.4. The water activity shift by temperature is mainly due to the change in water binding,

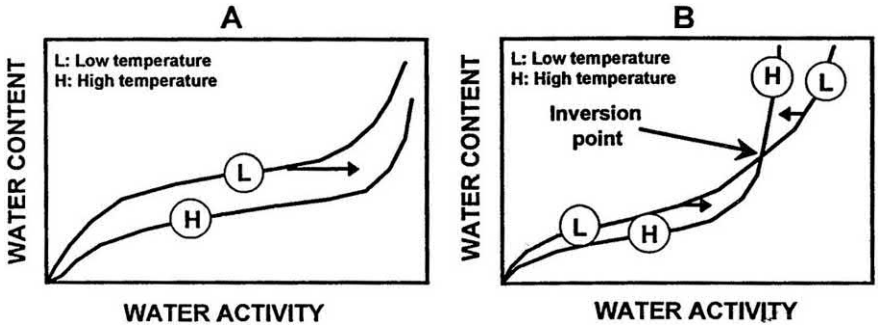

FIGURE 31.4 Water activity shift of food by temperature. (A) Shift without intersection. (B) Shift showing the point of intersection or inversion point.

dissociation of water, physical state of water, or an increase in the solubility of solute in water. The isotherm shift due to temperature can usually be estimated by the well-known Clausius–Clapeyron equation [45]:

$$\ln \frac{(a_w)_2}{(a_w)_1} = \frac{q + \lambda_w}{R}\left[\frac{1}{T_2} - \frac{1}{T_1}\right] \tag{31.2}$$

The slope of a plot of ln a_w versus $1/T$ should give the value of $(q + \lambda_w)/R$, where q is the excess heat of sorption (kJ/kg) and λ_w is the latent heat of vaporization for water (kJ/kg). It is widely accepted that an increase in temperature results in decreased equilibrium moisture content (Figure 31.4A). Tsami et al. [26] found similar results for the dried fruits up to a water activity of about 0.55–0.70. In that region the curves for several temperatures intersect. At water activity values higher than 0.7 there was an inversion of the effect of temperature (i.e., equilibrium moisture content increased with temperature) due to an increase in solubility of sugars in water. The intersection (or inversion) point depends on the composition of the food and the solubility of sugars [22]. For sultana raisin and currant, the inversion point was about 0.55, likewise, 0.65 for fig, 0.70 for prune, 0.75 for apricot (possessing the lowest sugar content of fruit) [26], 0.55 for quince jam (Ferbar brand), and 0.65 for quince jam (Tapada Nova brand) [46]. A similar intersection was also found for sultana raisin [47] and sugar alcohol [48]. Apple (a low-sugar fruit) does not show intersection [49]. For products with protein or starch content, there is also no intersection point with the increase of temperature [31].

31.3.3 Water Activity Break

In a pure component isotherm, the change of solute from the amorphous state to a crystal affects the isotherm. A break is observed in the isotherm, as shown in Figure 31.5. In some foods, one part of the solute (salts and sugar) is bound to a polymer (protein and starch), and the other part is crystalline or amorphous. Bound and free forms of solutes are in equilibrium, which is strongly dependent on the actual water activity. If the change in water activity takes place slowly, this equilibrium may be maintained, whereas during rapid changes nonequilibrium conditions are likely to be attained. Bound, crystalline, and amorphous solutes produce characteristic changes (i.e., break and shift) in the water sorption isotherm [50]. A typical curve showing a break is shown in Figure 31.4B, where a break is also observed due to the transformation of the solute from an amorphous to a crystalline state. This break was observed for sodium chloride–starch mixtures [51]; sucrose–starch mixtures [52]; sucrose albumin, and gluten mixtures [53]; and sucrose–casein mixtures [54].

31.3.4 Concept of the Local Isotherm and Different Types of Bound Water

Rockland [55] proposed the concept of the local (i.e., segment) isotherm to characterize the physical state or special type of water binding in foods. The local isotherm can be identified by graphical analysis of experimental sorption data according to Henderson's equation. The three localized isotherms may be distinguished by plotting experimental sorption data as ln[–ln(1 – a_w)] versus ln M_w. Three straight lines rather than a single straight line are observed, each being identified as a local isotherm. The three regions are identified as three types of water. Iglesias and Chirife [28] analyzed 235 isotherms based on this concept and concluded that although in a broad sense the local isotherms proposed by Rockland [55] may be related to the different modes of water binding, they cannot be used to give a precise and unequivocal definition of the physical state of water in foods. Moreover, the original Henderson equation should give only one curve and

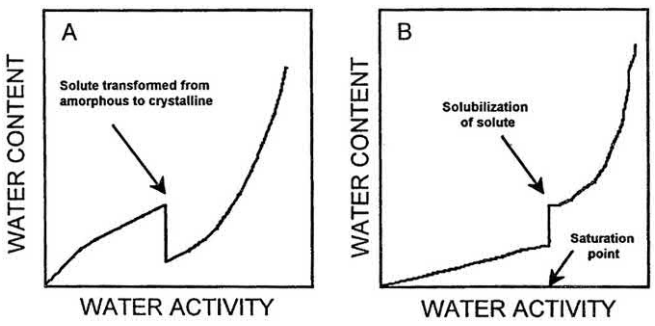

FIGURE 31.5 Water activity shift of food by physicochemical parameters. (A) Shift with solute transformation from amorphous to crystalline. (B) Shift with solubilization of solute.

segmented curves could present a poor fit. Caurie [56] used isotherm data fitted to the Caurie [57] model and determined three types of bound water along a decreasing energy gradient. They defined type I as nonfreezing strongly bound nonsolvent, type II as nonfreezing weakly bound solvent, and type III limited freezing loosely bound solvent. They argued their estimation was more justified over Rockland [55] since the proposed three types of bound water were estimated from only one linear fit of the data rather than segmentation of the isotherm.

31.3.5 Thermodynamic Properties Prediction

Thermodynamic properties such as the freezing point, boiling point, and heat of sorption can also be predicted from water activity. For example, the freezing point (Equation 31.3) and boiling point (Equation 31.4) can be estimated as follows [58]:

$$\ln a_w = 9.6934\times10^{-3}\Delta + 4.761\times10^{-6}\Delta^2 \qquad (31.3)$$

$$\ln a_w = 1.1195\times10^{-4}\Delta - 3.5127\times10^{-2}\Delta^2 \qquad (31.4)$$

where Δ is the freezing point depression or boiling point depression. The preceding equations could be very useful when measurement of the freezing point is very fast and easy. However, the freezing point is not easy to measure for low water content, especially unfreezable water.

31.3.6 Porous Structure Investigation

Water sorption can be influenced by the surface area and porosity of the food material. The characteristics of a material (e.g., porous or nonporous) can be determined from sorption isotherms. It is proposed in the literature that water activity could be used to calculate the food surface area as well as the pore size. However, Nagai and Yano [59] found the surface area and pore size calculated from the water sorption to be misleading. They suggested that water adsorption occurs not only on the surface, but mainly on the water-binding sites inside the structure, and these do not increase with an increase in surface area.

31.4 FACTORS AFFECTING WATER ACTIVITY

31.4.1 Food Components

Protein and starches adsorb much more water at low water activity than do fatty materials or crystalline substances like sugar. Pretreatment, such as heating, has little effect on proteins. On the other hand, such pretreatment increases the amount of water-impenetrable crystalline starch at the expense of amorphous starch. The smaller active site for adsorption means that less water could be adsorped [21]. Sugars and salts present a difficult problem because the change from an amorphous to a crystalline state occurs fairly rapidly at a normal temperature [60]. This change releases water, which may be picked up by other components if the sugar is present in a mixture such as dried milk. The material could become sticky and lumpy, making it undesirable. Salwin [61] observed that the equilibrium condition obtained is not an equal moisture content in all components of a multicomponent mixture but an equal activity.

31.4.2 Physicochemical State of Food Components

Many food components are in several states: crystalline solids, amorphous solids whether rubbery or glassy solids, aqueous solution, or bound to other components. Sorption in such systems is complex. Crystalline sugars adsorb very little water, but amorphous sugars adsorb substantially more water at the same conditions. The adsorption of water results in the breaking of some hydrogen bonds and an increase in the mobility of sugars molecules, resulting eventually in the sugars transforming to the crystalline state. In this process, sugar loses water [62]. However, the sugar–polymer interaction and physical state play an important role in separating out water from the system. Gelatinization followed by freeze-drying results in only minor differences in water-binding behavior up to water activity 0.94; above 0.95 the gelatinized samples adsorb considerably more water [63]. Saltmarch and Labuza [64] studied the effects of water activity and temperature on the transition of lactose from the amorphous to the crystalline state. Results from scanning electron microscopy indicated that lactose crystallized at 0.40, 0.33, and 0.33 water activity after one week at 25°C, 35°C, and 45°C, respectively. Water activity also influences protein conformation. The annealing effect of water, time, and temperature can alter the structural and functional properties of cereal starch (Figures 31.6 and 31.7). When crystalline starch is transformed into an amorphous form, polar sites are developed in the starch molecule, which could form hydrogen bonds with water molecules [65].

31.4.3 Porous Structure of Foods

Structure or pore size and distribution of materials may also affect the water activity, especially sharply increasing in the region in the isotherm at higher water activity. In this region, pores are expanded and water could adsorb in the capillary region.

31.4.4 Temperature

Above freezing temperature, the isotherm shift due to temperature can be estimated by the Clausius–Clapeyron equation as discussed earlier.

Information in the literature on water activity of the frozen state below freezing is limited [18]. The vapor pressures of animal tissues over the temperature span of –26°C to –1°C ranged from 13% to 20% lower than those of pure ice at the same temperature [66, 67]. Other researchers demonstrated that the vapor pressures of frozen biological materials were equal to the vapor pressure of ice at the same temperatures [68–71]. Water activity values at subfreezing temperatures can be calculated (rather than measured) as [18]

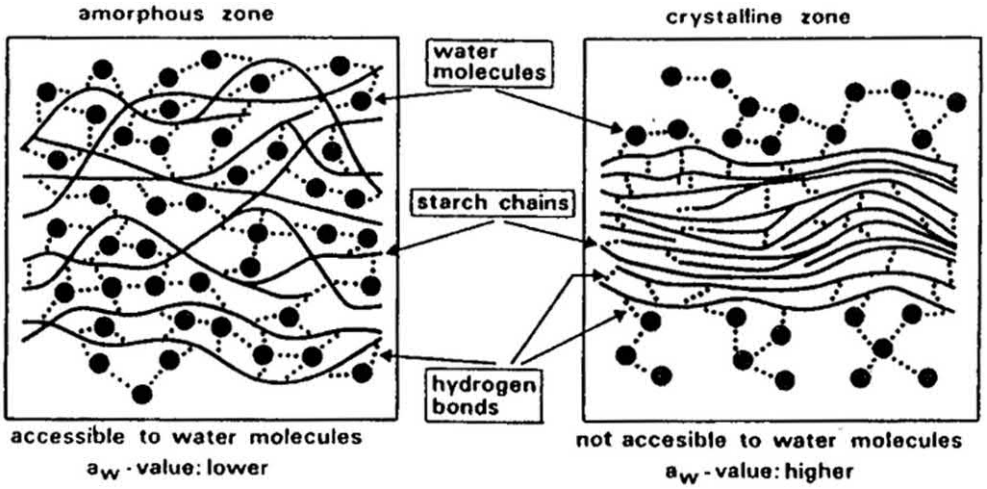

FIGURE 31.6 Schematic model of starch structure during amorphous and crystalline state. (From Munzing [65].)

$$a_w = \frac{\text{Vapor pressure of solid water (ice)}}{\text{Vapor pressure of liquid supercooled water (not ice)}} \tag{31.5}$$

The equation indicates that water activity does not depend on the composition, but only on the temperature. In a two-phase system (ice and solution) at equilibrium, the vapor pressure of solid water as ice crystals and the interstitial concentrated solution are identical, thus water activity depends only on the temperature, and not on the nature and initial concentration of solutes, present in the third or fourth phase (i.e., irrespective of the kind of food). This creates a basis to estimate the water activity of foods below freezing using Equation 31.5. Thus, Fennema [69] concluded that changes in properties can occur below freezing without any change in water activity. These include changes in diffusion properties, the addition of additives or preservatives, and disruption of cellular systems. The water activity data of ice from 0°C to –50°C is correlated with an exponential function as

$$a_w = 8.727\left[\exp\left(-\frac{595.1}{T}\right)\right] \tag{31.6}$$

where T is the temperature (K). The maximum error in prediction is 0.012 unit water activity and the average is 0.0066. The data of Fennema [72] are used to develop the preceding correlation.

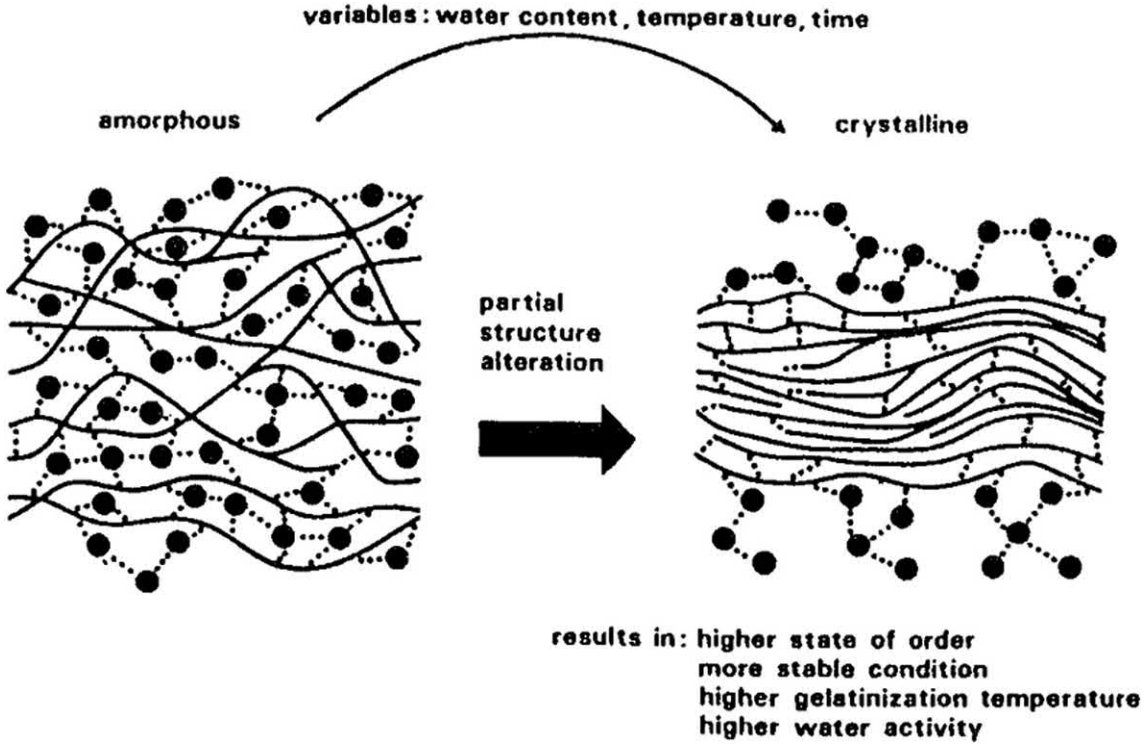

FIGURE 31.7 Annealing effects of starch induced by water, temperature, and time. (From Munzing [65].)

31.4.5 Pressure

The effect of pressure on the sorption isotherm is relatively small and negligible at reasonable pressure levels [14]. At constant moisture content, the variation of water activity with pressure can be derived thermodynamically as [14]

$$\ln\frac{a_2}{a_1} = \frac{\lambda_w}{\rho_w RT}[P_2 - P_1] \tag{31.7}$$

where a_1 and a_2 are the water activity at P_1 and P_2, R is the gas constant (82.05×10^{-3} m^3 atm/kg mole K or 8.314×10^3 m^3 Pa/kg mole K), T is the temperature (K), and P_1 and P_2 are total pressure (atm or Pa).

31.4.6 Surface Tension

The effect of capillary action on water activity can be estimated from the Kelvin equation as

$$a_w = \exp\left(-\frac{\Delta P V_m}{RT}\right) \tag{31.8}$$

For the spherical interface:

$$\Delta P = \gamma_s\left[\frac{1}{r_1} + \frac{1}{r_2}\right]\cos\theta \tag{31.9}$$

where ΔP is the pointing pressure (Pa), V_m is the liquid molar volume (18 m^3/kg mole), R is the gas constant (8.314 N m/kg mole K), T is the temperature (K), γ_s is the surface tension (N/m), cosθ is the contact angle, and r is the radius of curvature (m). If the droplet is spherical, then $r_1 = r_2$ and the preceding equation can be written as

$$a_w = \exp\left[-\frac{2\gamma\cos\theta V_m}{rRT}\right] \tag{31.10}$$

If the surface tension is reduced by a factor of 0.5, the ratio of water activities at the two conditions can be calculated using the preceding equation. The ratio is 0.995 at 20°C, thus the effect of surface tension cannot be measured. Chen and Karmas [73] reported that in the case of intermediate food solutions, water activity increased very little as the surface tension decreased. They suggested that ingredients that result in a reduction of surface tension should be avoided in order to attain low water activity. Alzamora et al. [74] found that surface tension did not appear to have any significant effect on water activity, at least within the range of apparatus error from 0.004 to 0.005 water activity unit.

31.5 WATER ACTIVITY PREDICTION MODELS

31.5.1 Raoult's Law

Ideally, water activity of pure water is 1 and solute depresses the water activity lower than 1. The water activity of an ideal solution could be predicted by Raoult's law, which indicates that water activity is equal to the mole fraction of water in a solution. It can be written as

$$a_w = m_w = \frac{X_w}{X_w + E X_s} \tag{31.11}$$

where a_w is the water activity, m_w is the mole fraction of water, X_w and X_s are the mass fractions of water and solute (g/g sample), and E is the molecular weight ratio (λ_w/λ_s) of water and solute. Raoult's law is not valid for macromolecular solute due to the very low value of E since it predicts nearly equal to 1 for all solute concentrations due to the high molecular weight of the macromolecules. Smith [75] mentioned that Raoult's law can be applied only to dilute solutions having water activity over 0.95. The nonideal behaviors are due to the molecular size and intermolecular force differences of solute and solvent, solvation effects, varied properties of water as affected by many factors, solute–solute and solute–solvent interactions, solute ionization, capillary action, and order of mixing in the cases of multiple solutes [7].

31.5.2 Activity Coefficient Model

In order to include the nonideality effect, Raoult's law is modified by introducing the water activity coefficient as

$$a_w = f_w m_w \tag{31.12}$$

where f_w is the water activity coefficient. The activity coefficient reflects the nonideal behavior of a molecule in solution and it is equal to 1 for dilute solution, when m_w tends to 0. It could be linked with different components as affected by differences in molecular sizes of water and solutes, and interaction among the functional group [76]. The activity coefficient values of food solutions are not readily available or predicted.

31.5.3 Flory–Huggins Model

The Flory–Huggins model, based on a statistical approach arising from entropy, is expressed for the nonideality due to the size differences between solute and solvent as [77, 78]

$$\ln a_w = \left[\frac{(\phi - 1)X_S}{X_w + \phi X_s}\right] + \ln\left[\frac{X_w}{X_w + \phi X_s}\right] \tag{31.13}$$

where ϕ is the ratio of the molar volumes of the solute and the solvent.

31.5.4 Schwartzberg Model

There are many models developed based on Raoult's law. Considering the effects of the nonsolvent water term in Raoult's law, Schwartzberg [79] developed the following model:

$$a_w = \frac{X_w - NX_s}{X_w - NX_s + EX_s} \tag{31.14}$$

where N is the nonsolvent water (g/g dry solids), and E is the molecular ratio of solutes and solvent. Solute interactions can enhance or decrease the effective amount of water acting as solvent, and it fits better to the nonelectrolyte solutions in some cases. However, considering a constant value of N over the entire concentration range is not reasonable, since interactions could vary with concentration [7]. A similar model is proposed by Chen [80] considering another additional parameter with E.

31.5.5 Norrish Model

Norrish [81] derived a model from thermodynamics for the prediction of water activity of nonelectrolyte solution as

$$a_w = m_w\left[\exp\left(\sum_i^n -\alpha_i m_{s,i}^2\right)\right] \tag{31.15}$$

where $m_{s,i}$ is the mole fraction of solute i, α_i is the Norrish model parameter for solute i, and n is the total number of solutes. For only one solute, the preceding equation can be written as

$$a_w = m_w\left[\exp\left(-\alpha m_s^2\right)\right] \tag{31.16}$$

The Norrish model is the most widely used model due to the availability of α values for a wide range of solutes. The values of α can be estimated from the slope of the plot ln (a_w/m_w) versus m_s^2 and this model considers constant slope within the whole range of concentration and the line should pass through the origin [82]. In many cases, the change in slope with concentration and deviation from the origin are observed, and two slopes and two intercepts are estimated diving one segment into two segments [82]. The values of α for different solutes are compiled in Tables 31.2 and 31.3.

The molecular weight of solute is needed to calculate the mole fractions. Therefore, the preceding equation could be extended into a two-parameter model for a multicomponent mixture or food when the effective molecular weight is not known [83]:

$$a_w = \frac{X_w}{X_w + E X_s}\left[\exp\left(-\alpha\left(1-\frac{X_w}{X_w + E X_s}\right)^2\right)\right] \tag{31.17}$$

where E is the molecular weight ratios of water and solute. Water activity data and mass fractions data could be used to estimate both parameters, α and E. Baeza et al. [84] tested Norrish model for correlating the water activity of highly concentrated solutions of sugars, polyols, and polyethylene glycols up to water activity 0.30. The variations of α for the same solution in Table 31.2 are due to the concentration range used to estimate the parameter. In the cases of polyethylene glycol 400 and 600, the Norrish model deviates significantly when considering high concentration (i.e., up to water activity 0.30). In this case, Baeza et al. [84] proposed the model as

$$a_w = m_w\left[\exp\left(-\alpha m_s^{\delta}\right)\right] \tag{31.18}$$

The exponent 2 in Equation 31.18 is now changed to a new parameter, δ. The values of α for polyethylene glycol 400 and 600 are observed as 1.49 and 1.98, while the values of δ are observed as 0.98 and 0.94, respectively [84].

31.5.6 Ross Model

Ross [85] derived an equation to estimate the water activity of a multicomponent mixture if the water activity of each component is available. It is under the assumption that

TABLE 31.2
Norrish Model Parameter for Nonelectrolyte Solution

Solute Type	Solute	α	Reference
Sugars	DE 42	5.31	Labuza [93]
	Galactose	2.24	Chirife et al. [108]
	Glucose	2.11	Chirife et al. [109]
	Glucose	2.92	Chirife et al. [109]
	Glucose	2.25	Labuza [93]
	Glucose	2.94	Bhandari and Bareyre [110]
	Fructose	2.15	Chirife et al. [109]
	Fructose	2.82	Chirife et al. [19]
	Fructose	1.77	Baeza et al. [84]
	Lactose	10.20	Labuza [93]
	Maltose	4.54	Labuza [93]
	Sucrose	6.47	Labuza [93]
	Sucrose	6.01	Baeza et al. [84]
	Xylose	1.54	Labuza (1984)
Polyols	Erythritol	1.34	Chirife et al. [108]
	Glycerol	1.16	Labuza [93]
	Glycerol	0.81	Baeza et al. [84]
	Mannitol	0.91	Labuza [93]
	Xylitol	1.66	Alzamora et al. [111]
	1,3-Butylene glycol	3.47	Alzamora et al. [111]
	Sorbitol	1.65	Labuza [93]
	Sorbitol	0.35	Baeza et al. [84]
	Propylene glycol	4.04	Alzamora et al. [111]
	Arabitol	1.41	Alzamora et al. [111]
	2,3-butylene glycol	4.78	Alzamora et al. [111]
Amides	Alanine	2.53	Labuza [93]
	Urea	2.02	Labuza [93]
	Glycine	0.87	Labuza [93]
	β-Alanine	2.52	Chirife et al. [108]
	Lactamide	0.71	Chirife et al. [108]
	Glycomide	0.74	Chirife et al. [108]
Others	Lactulose	8.00	Labuza [93]
	Lysine	9.30	Labuza [93]
	Ornithine	6.40	Labuza [93]
	Polyethylene glycol 400	26.60	Labuza [93]
	Polyethylene glycol 400	7.29	Baeza et al. [84]
	Polyethylene glycol 600	56.00	Labuza [93]
	Polyethylene glycol 600	12.88	Baeza et al. [84]
	Proline	3.90	Labuza [93]

TABLE 31.3
Norrish Model Parameter for Electrolyte Solution

Solute Type	Solute	α	Reference
Acid	α-Amino-n-butyric acid	2.59	Chirife et al. [108]
	α-Aminobutyric acid	2.57	Labuza [93]
	Citric acid	6.17	Labuza [93]
	Lactic acid	1.59	Labuza [93]
	Malic acid	1.82	Labuza [93]
	Tartaric acid	4.68	Labuza [93]
Salt	Potassium chloride	10.81	Labuza [93]
	Sodium chloride	17.48	Labuza [93]
	Calcium chloride	86.68	Bui et al. [112]

solute–solute interactions cancel on average and the equation could be written as

$$a_w = a_1 a_2 a_3 \ldots\ldots\ldots\ldots a_n \tag{31.19}$$

where a_1, a_2, and a_3 are the water activities of the individual components based on the total water in the system. Lilley [86] attempted to point out the apparent weakness of Caurie's equation and proposed his development by pointing out a series of factors: size differences between solute(s) and solvent, solvent binding, solute interactions, solvation, and volume differences. The equation is proposed considering the heterotactic term from the binary solutions [87]:

$$a_w = \frac{a_{12}\, a_{23}\, a_{13}}{a_1\, a_2\, a_3} \tag{31.20}$$

31.5.7 Caurie Model

The interaction terms are included in the Ross model. Caurie [88] derived an equation from the Gibbs-Duhem equation to predict the water activity of solution with mixed solutes:

$$a_w = a_1 a_2 a_3 - \left[\frac{n\left(\omega_1\omega_2 + \omega_1\omega_3 + \omega_2\omega_3\right)}{55.5^2} + \frac{(n+1)\omega_1\omega_2\omega_3}{55.5^2} \right] \tag{31.21}$$

where ω is the molality of solute, and n is the number of components in the mixture. The second and third terms of Equation 31.21 determine the interactions among solution components. It is the advance of the Ross equation considering the nonideal behavior of the solution.

31.5.8 BET Model

In the case of foods, the moisture sorption isotherms are mainly modeled with the Brunauer–Emme–Teller (BET) [89] and Guggenheim–Andersen–de Boer (GAB) [90–92] models. The BET equation is

$$M_w = \frac{M_b\, B\, a_w}{\left(1-a_w\right)\left[1+\left(B-1\right)a_w\right]} \tag{31.22}$$

where M_b is the BET monolayer water content (g/g dry-solids), M_w is the total water content (g/g dry-solids), a_w is the water activity, and B is a constant related to the net heat of sorption. The data of the moisture sorption isotherm within the water activity of 0.05 and 0.55 are used to fit the theoretical BET model to determine the parameters M_b (i.e., monolayer moisture content) and B [21]. The parameters of BET can be estimated graphically from the straight line by plotting $[a_w/(1–a_w)M_w]$ versus a_w and from the slope $[(B – 1)/M_b\, B]$ and intercept $[1/M_b\, B]$ the parameters can be estimated. The BET monolayer values showed a decreasing trend with the increasing equilibrium temperature. The value of B indicates how strongly bound the water in the solid matrix and can be related to temperature as

$$B = \gamma\left[\exp\left(-\frac{Q_s}{RT}\right)\right] \tag{31.23}$$

where Q_s is the excess heat of sorption (kJ/kg) and γ is the pre-exponent factor. The monolayer value is generally at a water activity of 0.2 to 0.4 [93]. In addition, the BET monolayer calculation is an effective method for estimating the amount of bound water to specific polar sites in dehydrated food systems [94]. The BET monolayer values usually vary from 0.01 to 0.14 (dry basis) in the case of foods and food components. Macromolecules, such as starch, protein, and agar usually have higher BET monolayers, whereas high fat content foods, such as avocado, peanuts, and whole milk, showed lower monolayers. Iglesias and Chirife [28] found that monolayer values decreased significantly with increasing temperature after studying 100 foods and food components. This may be due to the thermodynamics where higher temperatures increase the escaping tendencies of gas molecules.

31.5.9 GAB Model

Anderson [90] modified the BET equation from multilayers for strengthening the physical meaning and the fitting ability; however, Anderson's equation was later derived kinetically by DeBoer [91] and statistically by Guggenheim [92]. This equation was later named the GAB equation, and it is now one of the most popular and widely used for foods [12]. The GAB equation is

$$M_w = \frac{M_g\, C\, K\, a_w}{\left(1-K\,a_w\right)\left[1-K\,a_w+C\,K\,a_w\right]} \tag{31.24}$$

where M_g is the GAB monolayer moisture content (g/g dry-solids), C is a constant related to the monolayer heat of sorption, and K is a factor related to the heat of sorption of the multilayer. The parameters C and K can be correlated as the Arrhenius equation as

$$C = C_o \exp\left(\frac{\Delta H_c}{RT}\right) \tag{31.25}$$

$$K = K_o \exp\left(\frac{\Delta H_k}{RT}\right) \tag{31.26}$$

where T is the temperature (K), R is the universal gas constant (8.314 kJ/mole K), and ΔH_c and ΔH_k are the heat of sorption functions (kJ/mole). The GAB isotherm equation is an extension of the BET model considering modified properties of sorbate in the multilayer region and bulk water using the third constant K. It is valid up to water activity 0.9, while BET is valid up to water activity 0.55. The parameters of the GAB model are usually estimated by nonlinearizing regression and linear multiple regression after parabolic transformation of the nonlinear Equation 31.24.

31.5.10 Regression Parameters of BET and GAB

The BET and GAB parameters of foods are compiled in the literature for different types of foods [7]. Rahman and Al-Belushi [12] provided a comprehensive discussion on the physical meaning of the BET and GAB parameters. It is important to understand the regression methods and the physical meaning of the parameters before their uses in different applications. The convergence strongly depends on the initial values used in nonlinear regression. Two important points need to be considered while selecting the isotherm models. First is the accuracy of prediction, and the other is the physical meaning of the model's parameters. The accuracy indicates the mathematical representation of the experimental data, but physical meaning indicates the valid explanation of the physicochemical process (i.e., if parameter could be experimentally determined and compared) [12].

The BET monolayer is commonly used as an effective method for estimating the amount of bound water. However, the physical meaning of heat of sorption (i.e., derived from B) may not be considered a very acceptable physical meaning. This is due to many reasons. One is the limited validity of the relationship of B with the heat of sorption due to the number of assumptions used in the BET equation [28]. In many instances, the heat of binding of water from temperature dependency was completely wrong [28], and it was difficult to explain the negative values B in many instances [12, 95–98]. Another reason is the explanation of the physical meaning of very high estimated values (i.e., in the order of 10^6 to infinity). For example, Young [99] found the infinity value for B in the case of peanuts. In the case of hazelnut, the value of B was found in the order of 10^6 at 3°C, whereas at 10 and 30°C the values were in the order of 100 and 10 [100]. However, the lack of reliability of the energy parameter B does not preclude the use of the BET equation for determination of the monolayer value [28]. It is well documented for its physical meaning and validity for its monolayer value [12].

The GAB model is found accurate up to water activity 0.90 and it is now well accepted that the GAB model is one of the most accurate models for predicting isotherms of foods. The physical meaning of GAB may not be valid in all cases, although it is emphasized in the literature [12]. The main reasons are discussed as follows. In many instances, completely unreliable monolayer values are observed even when it gives the most accurate prediction [101]. Theoretically, the values of K should be less than unity [102]. The GAB model described well sigmoidal isotherms when parameters are kept as $0.24 < K < 1$ and $5.67 \leq C \leq \infty$, and outside these regions the isotherm is no longer sigmoid or the high estimation of monolayer value (i.e., ±16) [103]. In addition, these regions fulfill the requirement of BET. Commonly, different regression methods and optimization techniques could be used to estimate the parameters, and this may result in varied values due to many local optimized regions. In addition, the estimated parameters could be varied based on initial values used in nonlinear regression. In many instances, it is very difficult to obtain convergence for the C value and estimated negative values of C [12]. However, these are the generic problems when the theoretical model is extended to fit the experimental data and attempted to be related to the physicochemical process [104]. It is important to point out that the BET monolayer has more physical meaning and acceptability to be used for food stability compared to the GAB monolayer, although GAB provided better mathematical prediction of isotherm over the wide range of water activity as discussed by Rahman and Al-Belushi [12].

31.5.11 Grover Model

Grover [105] used actual measured data for the water activity of confectionery solids to develop a polynomial expression:

$$a_w = 1.04 - 0.10\mu + 0.0045\mu^2 \tag{31.26}$$

$$\mu = \sum_i^n \frac{\beta_i}{W_i} \tag{31.27}$$

where W_i is the moisture content based on component i mass (kg water/kg solute), and β_i is the constant for the ith component. The values of β_i are given in Table 31.4.

31.5.12 Composition-Based Models

Gabriel [106] developed the water activity of glucose syrup as a function of pH and degrees Brix using a second-order polynomial equation. Moreira et al. [107] proposed an algorithm of prediction of the water sorption isotherm considering soluble components (i.e., glucose, fructose, sucrose, and salt) and insoluble components (i.e., protein, fiber, and starch) of food and temperature (20–40°C). There are many models available in the literature, which may explain the physicochemical processes; however, at the end, the parameters are not available for a wide variety of foods.

TABLE 31.4
Grover Model Parameter for Different Solutes

Component	β_i
Sucrose	1.0
Lactose	1.0
Invert	1.3
42 DE	0.8
Protein	1.3
Starch	0.8
Gums	0.8
Acid	2.5
Glycerol	4.0
Salt	9.0
Fat	0.0

Source: Baeza et al. [84].

31.6 CONCLUSION

Water activity is defined from fundamental thermodynamics and used to determine food stability during processing and storage. It indicates the state of water foods, which indicate the reactivity of water in physical changes, chemical reactions and availability to the microorganisms. The fundamental aspects of the isotherm (i.e., hysteresis, water activity shift and break, local isotherm concept, and relationships with other colligative properties), factors affecting water activity (i.e., food components, states or phases, structure, temperature, pressure, and surface tension), and their prediction models are explained in this chapter.

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32 Water Activity and Food Preservation

Mohammad Shafiur Rahman and Theodore P. Labuza

CONTENTS

32.1 INTRODUCTION

The water activity concept has been widely used in food processing and preservation, as Scott [1] emphasized that active water is much more important to the stability (i.e., reactivity) of food than the total amount of water present. It is possible to develop generic rules or limits for food stability based on water activity, and microbial growth, chemical reactions, and physical changes can be correlated with water activity. This chapter discusses the effects of water activity on microbial growth, fat oxidation, nonenzymatic and enzymatic browning, vitamin losses, and texture.

32.2 MONOLAYER CONCEPT

The moisture sorption isotherm could predict that water bonds strongly (i.e., Brunauer–Emmett–Teller (BET)-monolayer) to the polar sites of a food matrix (Figure 32.1). It is considered that physical and chemical changes are minimum at its BET monolayer, thus foods are most stable at this moisture content. Water at this point or below is considered strongly bound and microorganisms are unable to use it; and, in general, reactants are also unable to use it for any reaction to be progressed.

32.3 FOOD STABILITY DIAGRAM

The moisture sorption isotherm is an extremely valuable tool for food scientists because of its usefulness in predicting food stability. Most foods have a critical moisture content below which the rate of quality loss is negligible. Quality is understood to include growth and toxin production by microorganisms as well as chemical deterioration and decrease of sensory intensity, such as crispness, hardness, caking, texture, color, flavor, and aroma [2]. A general food stability map is presented in Figure 32.2 [3, 4]. As discussed by Labuza [2], the rate of quality loss begins to increase above water activity 0.2–0.3 for most chemical reactions (Figure 32.2). At this water activity,

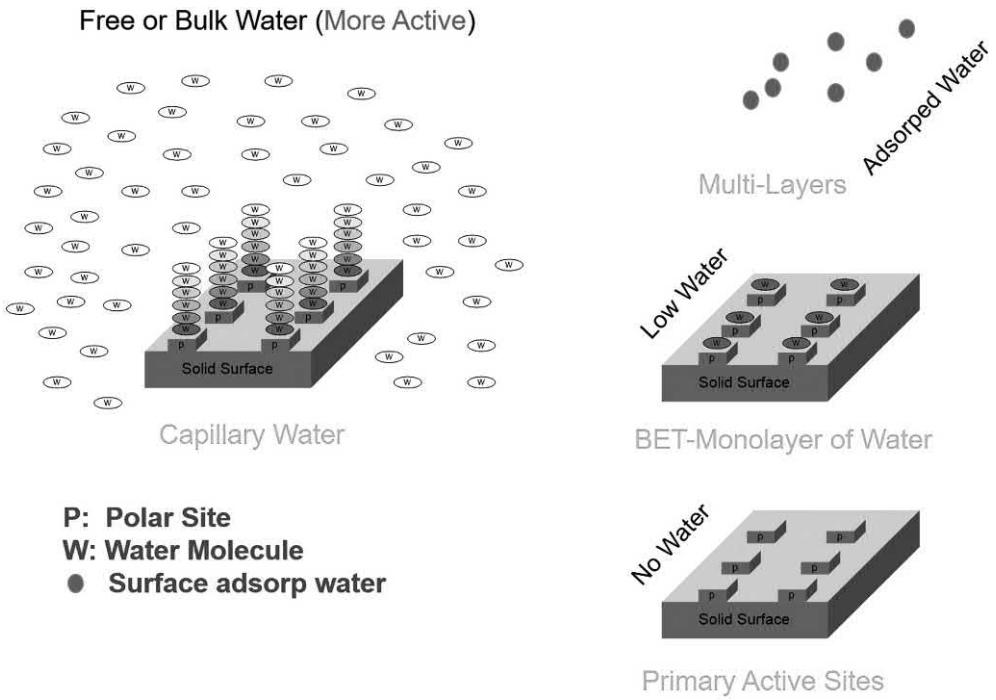

FIGURE 32.1 Different types of water on a surface (i.e., polar sites, monolayer, multiplayer, bulk or free water, and surface adsorbed water). (Adapted from Rahman [148].)

the amount of water adsorbed on surfaces and in capillaries is enough to affect the overall dielectric properties, such that the water can now behave as a solvent. Thus, chemical species can dissolve, become mobile, and are reactive.

The higher the water activity, the faster the reaction rate because of the greater solubility and increased mobility of the reactants. However, at some higher water activity no more species dissolve and therefore an increase in water activity decreases the concentration of the reacting species. Since the rate of a reaction is proportional to concentration on a molecular basis, the rate should reach a maximum and then fall as in Figure 32.2. Between this maximum and the monolayer, a semi-log plot of rate versus water activity generally results in a straight line. For most dry foods, an increase of 0.1 water activity units in this region decreases shelf life two to three times [2].

32.4 MICROBIAL MINIMUM WATER ACTIVITY LIMIT

A microorganism or group of microorganisms can no longer reproduce below a minimum water activity limit. Hypothetical curves showing the effects of water activity on the growth curves are presented in Figure 32.3. The initial portion of this growth curve is composed of a lag phase during which the physiological machinery is being created for later growth. The lag period is increased with an increase in solutes content or a decrease in water activity. The growth or logarithmic phase also affected by water activity is shown in Figure 32.3 [5]. Secondary metabolites produced by some microorganisms are highly toxic and/or carcinogenic to humans. The factors that affect spore formation can influence the formation of these metabolites. Beuchat [6] summarized the effects of water activity on spore formation and germination as well as toxin production by microorganisms commonly associated with foods and food spoilage.

Minimal water activity values for growth and toxin production by microorganisms of public health significance are listed in Tables 32.1–32.8. The concern of food safety increases with increasing water activity. The water activity values of some foods are their susceptibility to spoilage microorganisms are shown in Table 32.8 [6]. There is a critical water activity below which no microorganisms can grow. For most foods, this is in the 0.6–0.7 water activity range. Pathogenic bacteria cannot grow below a water activity of 0.85–0.86, whereas yeast and molds are more

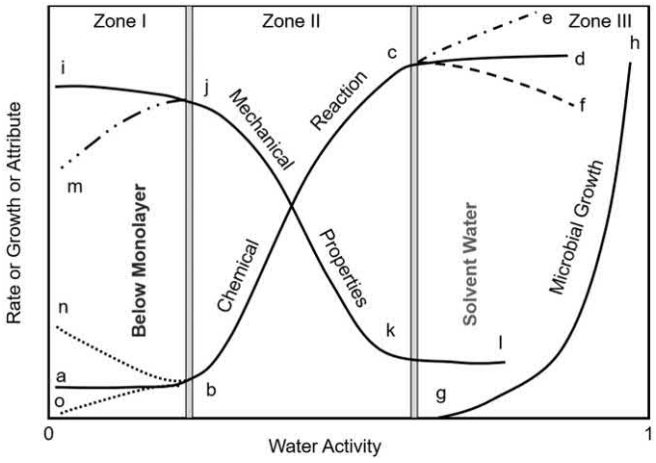

FIGURE 32.2 Food stability as a function of water activity. (From [4, 148].)

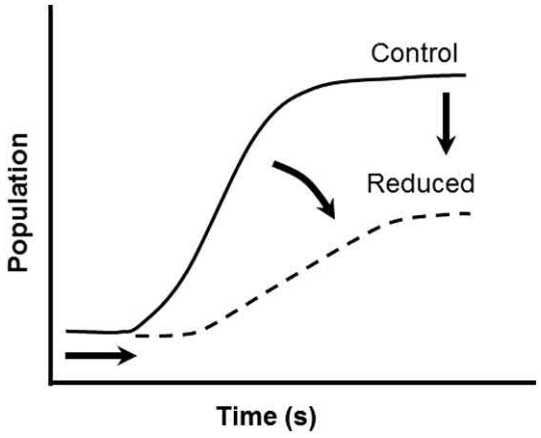

FIGURE 32.3 Hypothetical curves showing the effects of water activity reduction on growth bacteria. (From Troller [5].)

tolerant of a reduced water activity of 0.80, but no growth usually occurs below a water activity of about 0.62 [7]. The critical limits of water activity may also be shifted to higher or lower levels by other factors, such as pH, salt, antimicrobial agents, heat treatment, and temperature of storage. The rate of microbial death during frozen storage can be reduced by a decrease in temperature and no fluctuation in temperature [8]. The microbiological quality of frozen foods could be improved by initial storage of foods at –10°C (a_w = 0.90), to reduce the number of undesirable organisms followed by freezing at very low temperature (i.e., –30°C) [9]. In the case of selected penicillia, spores produced on media at 0.99 water activity appeared more heat resistant compared to those produced at 0.88 water activity [10]. All treated spores were more sensitive to benzoate and sorbate but more resistant to cycloheximide. McClure [11] provided review on the effect of water activity to the growth of microorganisms.

The growth and aflatoxin B_1 production in 20 isolated *Aspergillus flavus* were studied as a function of water activity and storage temperature [12]. The critical water activity (a_c) was determined by probability model considering 39 days of storage and it was observed that a_c varied with storage temperature. For examples, the critical water activity limit was 0.85 at 25–30°C, white it was increased to 0.94 at 15°C and 0.93 at 40°C. Garcia-Cela et al. [13] studied the secondary metabolites in stored natural wheat and inoculated with *Fusarium graminearum* as a function of water activity (0.95–0.90) and temperature (10–25°C). They observed that both temperature and water activity affected the numbers and types of metabolites. Kapetanakou et al. [14] observed the growth rate and ochratoxin A production by *Aspergillus carbonarius* as a function of water activity (0.90–0.99), temperature (15–25°C) and structure formed by gelatin (0–20%, w/v). They observed all three factors affected growth rate nonlinearly and they modeled a quadratic form equation.

32.5 WATER STRESS (DESICCATION) AND ADAPTATION MECHANISMS

Microorganisms are adapted in a wide range of environments, and their genetic and physiological adaptability enables them to withstand harsh environmental factor(s), single or in combination [15]. The ability to adapt and persist depends on the sensing and responding to physicochemical and other environmental changes. It requires profound physiological changes in the sessile (i.e., multicellular aggregates adhering to biotic or abiotic surfaces and/or to each other), planktonic (i.e., free suspension in liquid medium), and biofilm (i.e., microcolonies of bacteria associated with an inert surface attached by a matrix of complex polysaccharide-like material and enclosed nutrients with trapped microorganisms). A biofilm is a unique environment that microorganisms generate for themselves, and cells apply their regulations of gene expression in response to various signals [16]. *Listeria monocytogenes* in a biofilm survived up to 49 days against a desiccated environment (43% RH and 15°C) [17].

Recently, Esbelin et al. [18] reviewed the adaptation mechanisms of bacteria during desiccated bacteria. The degree of adaptation varied with the type of bacteria. For examples, *Deinococcus radiodurans* [19] and *Mycobacterium* [20] are extremely resistant to prolonged desiccation, whereas

TABLE 32.1
Minimal a_w for Growth and Toxin Production by Bacteria of Public Health Concern

	Minimal Water Activity		
Bacteria	**Growth**	**Toxin Production**	**Toxin**
Bacillus cereus	0.93–0.95	—	—
Clostridium botulinum	0.93–0.95	0.94-0.95	Type A
	0.93–0.94	0.94	Type B
	0.95–0.97	0.97	Type E
Clostridium perfingens	0.93–0.95	—	—
Salmonella spp.	0.92–0.95	—	—
Staphylococcus aureus	0.86–0.87	0.87–0.90	Enterotoxin A
	0.86–0.87	0.97	Enterotoxin B
Vibrio parahaemolyticus	0.94	—	

Source: Beuchat [149].

TABLE 32.2
Minimal a_w for Growth of Foodborne Pathogens in Laboratory Media at Optimum pH and Temperature

Pathogen	Minimal a_w	Pathogen	Minimal a_w
Campylobacter jejuni	0.990	*Salmonella* spp.	0.940
Aeromonas hydrophilia	0.970	*Escherichia coli*	0.935
Clostridium botulinum E	0.965	*Vibrio parahaemolyticus*	0.936
Clostridium botulinum G	0.965	*Bacillus cereus*	0.930
Shigella spp.	0.960	*Listeria monocytogenes*	0.920
Yersinia enterocolitica	0.960	*Staphylococcus aureus* (anaerobic)	0.910
Clostridium perfringens	0.945	*Staphylococcus aureus* (aerobic)	0.860
Clostridium botulinum A and B	0.940		

Source: Chirife [150].

TABLE 32.3
Sodium Chloride versus Glycerol in Minimum Water Activity Supporting Growth of Pathogenic Bacteria

	a_w Adjusted	
Bacteria	**Sodium Chloride**	**Glycerol**
Clostridium botulinum E	0.966	0.943
Clostridium botulinum G	0.966	—
Escherichia coli	0.945	0.940
Clostridium perfringens	0.945	0.930
Salmonella spp.	0.941	—
Clostridium botulinum A and B	0.940	0.930
Vibrio parahaemolyticus	0.932	0.911
Bacillus cereus	0.930	0.920
Listeria monocytogenes	0.920	0.900
Staphylococcus aureus	0.860	0.890

Source: Chirife et al. [147].

TABLE 32.4
Minimal Water Activity for Growth of Pathogenic Bacteria[a]

Bacteria	NaCl	KCl	Sucrose	Glucose
Listeria monocytogenes	0.920	—	0.920	—
Vibrio parahaemolyticus	0.935	0.936	0.940	—
Clostridium botulinum G	0.965	—	0.965	—
Clostridium botulinum E	0.972	0.972	0.972	0.975
Clostridium perfringens	0.945	—	—	0.946
Staphylococcus aureus	0.864	0.864	0.867	—

Source: Chirife [134].

[a] In laboratory media, water activity adjusted with salts (NaCl and KCl) or sugars (sucrose and glucose).

Neisseria gonorrhoeae [21] can survive only a short period of desiccation. called Xerophiles are microorganisms that can grow and multiply in an environment of extremely low water activity, even lower than 0.80 [22]. Worldwide, between the years 2007 and 2012, 7315 cases of bacterial infection and 63 deaths due to consumption of contaminated low water activity foods were reported [23].

32.5.1 Heat Resistance in Desiccation

Thermal resistance of microorganisms increases with the decrease of water activity and *D*-value is commonly used to explain the thermal resistance [24–26]. For example, it was observed that *D*-values of *Salmonella enteritidis* PT 30 increased from 0.96 to 6.97 min as water activity values decreased from 0.946 to 0.720 [27]. A more detailed review is given by Zhang et al. [28]. Pathogens exhibit resistance at decreasing water activity and fat protected against inactivation [29]. Considering *Salmonella*, other factors include types of solutes in the matrix, acidity, growth medium, stage of cell growth, stress prior to heating, species, and strain [30].

Zhang et al. [28] identified that nonisothermal heating (i.e., heating rate) also affects the heat resistance in addition to isothermal heating temperature. Considering three strains of *Staphylococcus aureus* ATCC 25923 in walnut shell powder (moisture: 18.0 g/100 g sample), they showed that these became more thermo-tolerant at lower heating rate. The *D*-value decreased with decreasing water activity and heating rates (<1°C/min). A significant increase in heat resistance was observed at heating rates of 0.2 and 0.5°C/min, whereas no effect at 1, 5, and 10°C/min. A rapid reduction was achieved at elevated temperature from 26 to 56 at a heating rate of 0.1°C/min. Thus, appropriate moisture, temperature, and heating rate are required for thermal inactivation.

TABLE 32.5
Minimal a_w for Growth of and Toxin Production by Molds of Public Health Concern

	Minimal Water Activity		
Mold	**Growth**	**Toxin Production**	**Toxin**
Alternaria alternata	—	>0.90	Altenuene, alternariol, alternariol monomethyl ether
Aspergillus flavus	0.78–0.80	0.83–0.87	Aflatoxin
Aspergillus parasiticus	0.82	0.87	Flatoxin
Aspergillus orchraceus	0.77–0.83	0.83–0.87	Ochratoxin
Byssochlamys nivea	0.84	—	—
Penicillium cyclopium	0.81–0.85	0.87–0.90	Ochratoxin
Penicillium viridicatu	0.83	0.83–0.86	Ochratoxin
Penicillium ochraceus	0.76–0.81	0.80–0.88	Penicillic acid
Penicillium cyclopium	0.82–0.87	0.97	Penicillic acid
Penicillium martensii	0.79–0.83	0.99	Penicillic acid
Penicillium islandicum	0.83	—	—
Penicillium urticae	0.81–0.85	0.85–0.95	Patulin
Penicillium expansum	0.83–0.85	0.99	Patulin
Stachybotrys atra	0.94	0.94	Stachybotrym
Trichothecium roseum	0.90	—	Trichothecene

Source: Beuchat [6].

TABLE 32.6
Water Activity of Some Foods and Susceptibility to Spoilage by Microorganisms

Range of a_w	Microorganisms Generally Inhibited by Lowest a_w in This Range	Examples of Foods Generally within This Range of a_w
1.00–0.95	*Pseudomonas*, *Escherichia*, *Proteus*, *Shigella*, *Klebsiella*, *Bacillus*, *Clostridium perfringens*, some yeasts	Highly perishable foods (fresh and canned fruits, vegetables, meat, fish) and milk; cooked sausages and breads
0.95–0.91	*Salmonella*, *Vibri paahaemolyticus*, *C. botulinum*, *Serratia*, *Lactobacillus*, *Pediococcus*, some molds, *Rhodotorula*, *Pichia*	Some cheeses (Cheddar, Swiss, Muenster, provolone), cured meat, some fruit juice concentrates
0.91–0.87	Many yeasts (*Candia*, *Torulopis*, *Hansenula*), *Micrococcus*	Fermented sausage (salami), sponge cakes, dry cheeses, margarine
0.87–0.80	Most molds (mycotoxigenic pencillia), *Staphylococcus aureus*, most *Saccharomyces* (billii) ssp., *Debaryomyces*	Most fruit juice concentrates, sweetened condensed milk, chocolate syrup, maple and fruit syrup, rice, pulses, fruit cakes, country-style ham, fondants, high sugar cake
0.80–0.75	Most halophilic bacteria, mycotoxigenic aspergilli	Jam, marmalade, marzipan, glace fruit, some marshmallows
0.75–0.65	Xerophilic molds (*Aspergillus chevalieri*, *A. candidus*, *Wallemia sebi*), *Saccharomyces bisporus*	Rolled oats, grained nougats, fudge, marshmallows, jelly, molasses, raw cane sugar, some dried fruits, nuts
0.65–0.60	Osmophilic yeasts (*Saccharomyces rouxii*), a few molds (*Aspergillus echinulatus*, *Monascus bisporus*)	Dried fruits, some toffees and caramels, honey
0.50	No microbial proliferation	Noodles, spaghetti, dried spices
0.40		Whole egg powder
0.30		Cookies, crackers, bread crusts
0.20		Whole milk powder, dried vegetables, corn flask, dehydrated soup, some cookies, crackers

Source: Beuchat [6].

TABLE 32.7
Compatible Protoplasmic Solutes in Fungi

Solute	Genus	Solute	Genus
Mannitol	*Geotrichum* *Platymonas* *Aspergillus* *Dendryphiella* *Penicillium*	D-Galactosyl-(1,1)-glycerol Glycerol	*Ochromonas* *Chlamydomonas* *Aspergillus* *Dunaliella* *Saccharomyces*
Cyclohexanetetrol	*Monochrysis*		*Debaromyces*
Arabitol	*Dendryphiella* *Saccharomyces*	Erythritol	*Aspergillus* *Penicillium*
Sorbitol	*Stichococcus*		

Source: Brown and Simpson [51].

TABLE 32.8
Minimum water Activity Values for Enzymatic Reactions in Selected Food Systems

Product/ Substrate	Enzyme	T (°C)	Water Activity Threshold
Grains	Phytases	23	0.90
Wheat germ	Glycoside-hydrolases	20	0.20
Rye flour	Amylases	30	0.75
	Proteases	—	—
Macaroni	Phospholipases	25–30	0.45
Wheat flour dough	Proteases	35	0.96
Bread	Amylases	30	0.36
	Proteases	—	—
Casein	Trypsin	30	0.50
Starch	Amylases	37	0.40/0.75
Galactose	Galactosidase	30	0.40–0.60
Olive oil	Lipase	5–40	0.25
Triolein, triaurin	Phospholipases	30	0.45
Glucose	Glucose oxidase	30	0.40
Linoleic acid	Lipoxygenase	25	0.50/0.70

Source: Drapron [151].

Farakos et al. [29] determined that the Weibull model was the most accurate model to predict the survival of *Salmonella* as a function of temperature (21–80°C) and water activity below 0.6. The Weibull model is

$$\log N = \log N_o - \left(\frac{t}{\delta}\right)^{\beta} \tag{32.1}$$

where N and N_o are the population at any time and initial population, respectively; t is time (s); δ-value is the time required for first decimal reduction (minutes), and β-value is a fitting parameter that defines the shape of the curve. D-value is defined as

$$\log N = \log N_o - \frac{t}{D} \tag{32.2}$$

where D is the decimal reduction time (s) (D-value). Farakos et al. [29] developed predicted models for log δ and log βas

$$\log \delta = -0.10T - 4.34a_w + 9.91$$

$$\log \beta = -0.006T$$

The temperature varied from 10°C to 90°C and water activity from 0.1 to 0.7. Figures 32.4 and 32.5 show a high variability in log δvalues and log βvalues, and the influencing factors are temperature, water activity, types of products, and serotype [31].

32.5.2 Influx and Outflow through Membrane

For many years scientists believed these mechanisms relied mainly on the influx and outflow of small, charged inorganic particles, primarily the ions of sodium, potassium, hydrogen, and chloride. Cell physiologists are coming to appreciate that changes in a cell's volume compromise more than just the shape or even the integrity of a cell. Any imbalance in the number of dissolved particles between the interior and exterior of a cell can cause water either to rush in and burst the cell's membrane, or to seep out, causing the cell to shrink. In the case of hypertonic, the cells shrink, whereas in case of hypotonic, cells expand [32].

A decrease in water activity in the environment increases the osmotic stress to microbial cells because the cell always tries to maintain a slightly lower internal osmolality (i.e., water activity). This causes an influx of water into the cell to maintain surface integrity. It is the disruption of this process by solutes that leads to cell damage and death. In addition to osmotic stress, solutes may have other effects on microorganisms, including enzyme inhibition, cytoplasmic coagulation, and damage to the cell wall. Zhu and Dai [33] observed a cross-protection effect of *Escherichia coli* to antibiotic tetracycline and chloramphenicol at a high salt level. This protection originated from the increased AcrAB-TolC efflux pump expression level when high salt was applied. Water activity can explain only the osmotic stress. Genetic control is closely tied to the amino acid pool, especially betaine and praline, and the potassium level in the cell.

32.5.3 Adaptation (K^+ Transport Systems)

A variety of mechanisms may avoid water loss or gain from microorganisms [34]. Such mechanisms are reviewed by Troller [5]. Potassium ion is essential for cell turgor pressure recovery despite its toxicity [35]. Although potassium may or may not be the trigger that initiates the process of osmoregulation, its transport into the cell is the primary modulatory event [36]. Helmer et al. [37], in a series of experiments, demonstrated that at least two and probably four K^+ transport systems exist in *Escherichia coli*. The first is accomplished, in part, by a series of four high-affinity genes. The first three

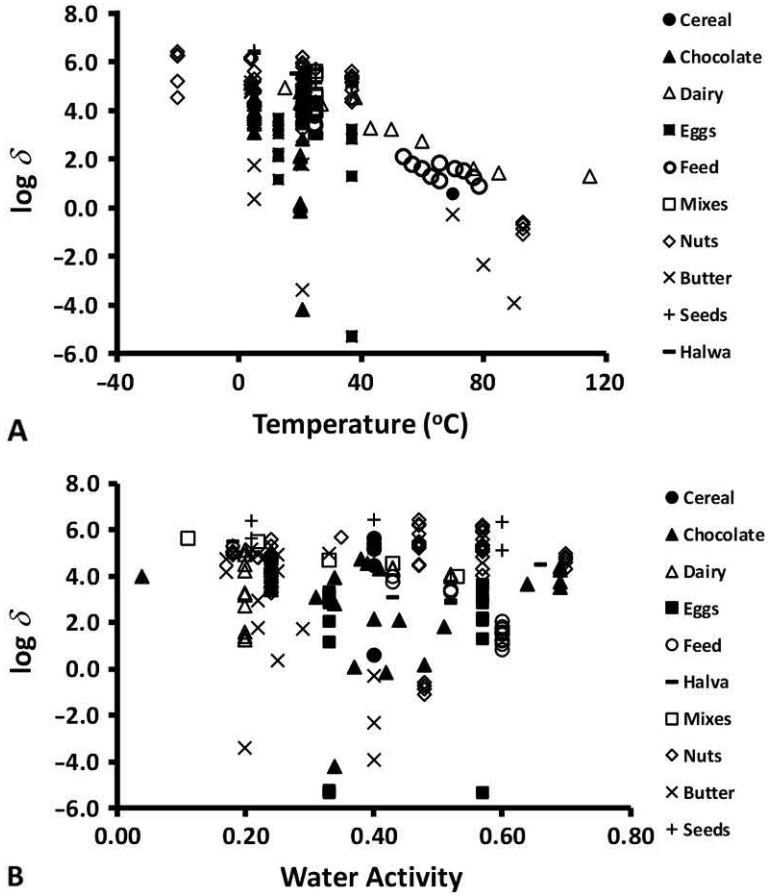

FIGURE 32.4 Log δvalues of *Salmonella* survival in various food products are plotted against (A) temperature and (B) water activity. (From Farakos et al. [31].) (Data for fat-containing products are in bold.)

genes in inner membrane proteins of various molecular weights act as gatekeepers. The fourth gene alters its conformation in a manner that permits and intensifies transcription to maintain cytoplasmic K^+ content [38].

The second system is constitutive and requires ATP and a proton motive force to supply energy for net K+ uptake. Helmer et al. [37] identified a proton motive force as supplying the primary energy to drive this reaction, whereas ATP supplies the energy to turn off K^+ transport. Another system, the K^+ export model, has only been postulated and is of some interest because of the potential existence of export-blocking proteins that might be synthesized by the cell in response

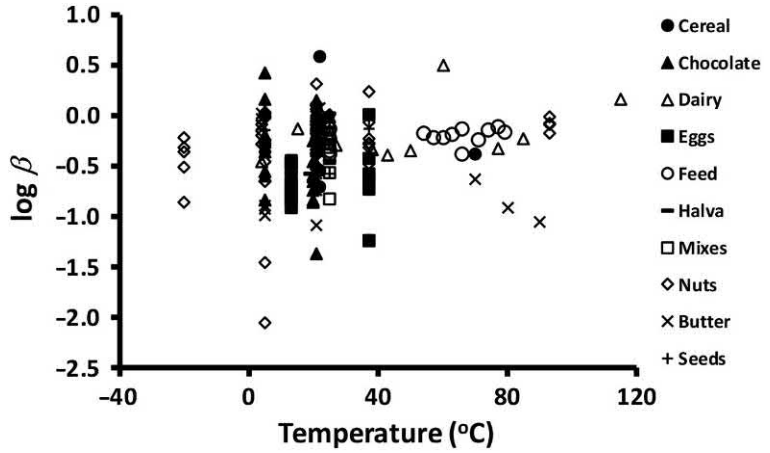

FIGURE 32.5 Log βvalues for survival of *Salmonella* serotypes in various food products plotted against temperature. (From Farakos et al. [31].)

to osmotic challenge. In this case, K^+ would not be pumped out of the cell but would be retained to trigger a metabolic response or to provide primary isoosmotic conditions across the membrane [5]. Bacteria imports K^+ through Kup, Trk, and Kdp transport systems [39].

32.5.4 Adaptation (Compatible Solutes)

The loss of turgor pressure increases the molecular crowding and is called the *water replacement hypothesis* [40]. In this process, the nonreducing disaccharides sucrose and trehalose are considered to preserve protein structures and preserve the membrane function by replacing bacterial membranous water [41]. The development of trehalose and sucrose hydrogen bond with the membrane phospholipids and replaces the water shell around macromolecules. This prevents the membranes to form gels [42, 43] and maintains the fluidity of the membrane [44]. In addition, sugar molecules protect the cells to form vitreous cytoplasmic matrix by chemical balancing the macromolecules when water is lost [45].

Christian [46] and Christian and Waltho [47] observed first that the growth of *Salmonella oranienburg* at low water activity was stimulated by the addition of the amino acid praline. They observed a reversal of plasmolysis when exogeneous proline was supplied to the bacteria growing at low water activity. Although uptake from the media may be one method of accumulating proline in response to water stress, most organisms appear to be able to synthesize proline. In fact, synthesis probably is the most common mechanism for accumulating proline in osmotically inhibited bacteria [48]. This is called *compatible solute*. A number of osmoregulatory solutes protect proteins against denaturation by heat [34]. Measures [49] and Gould and Measures [50] showed K^+ was required to maintain electrical neutrality or to balance the charges within cells exposed to environments with low water activity in which various amino acids, such as αketoglutarate and glutamic acid, accumulate intracellularly. The principal reaction involves the conversion of αketoglutarate to glutamic acid by glutamate dehydrogenase, an enzyme activated by K^+. Glutamic acid reduces the intracellular water activity to reverse plasmolysis by reducing relative amounts of K^+ and glutamate dehydrogenase. This leaves the cell at a balanced, osmotic null point by virtue of the increased glutamic acid pool. For some bacteria, the process stops at this point, but for other organisms, glutamic acid is converted to γaminobutyric acid or proline, neither of which are highly charged. Accumulation of high concentrations of glutamic acid would require concomitant acquisition of K^+ to keep the system at neutrality. This excessive amount of K+ could be detrimental to the organism and, at the very least, costly in terms of energy expenditure. Both γaminobutyric acid and proline are remarkably efficient at reducing intracellular water activity without interfering in the cell's metabolism and for this reason have been termed compatible solutes [51].

Compatible protoplasmic solutes in bacteria include glycylbetaine, proline, glutamic acid, γaminobutyric acid, and glycerol. Polyols of various types are compatible protoplasmic solutes in many fungi (Table 32.8). The uptake or synthesis of sugars, free amino acids, polyols, quaternary amines, sulfate esters, inositol phosphates, or mannosylglyceramides is common [52]. It is also known that the accumulation and production of xeroprotectants (e.g., trehalose) by some microbes and plants enables them to endure in extreme abiotic stresses [18]. Exactly how these solutes are able to avoid interference is not fully understood [5]. Gould [34] suggested that specific binding between solutes and intracellular enzymes is not the mechanism. Jones and Pollard [53] suggested that because these solutes may be excluded from the hydration sphere of proteins, the term benign solutes might more accurately describe the nonparticipatory nature of these materials. These steps (i.e., flashing out water, K+ transport, and compatible solutes) are shown in Figure 32.6.

32.5.5 Adaptation (Changing Cell Metabolism)

An important role of the membrane may be to exclude Na^+, which, if permitted to enter the cell, can quickly inactivate a number of vital enzymatic systems. Kanemasa et al. [54] attributed the barrier properties of *Staphylococcus aureus* membranes to Na^+. They found that Na^+ alters the types and amount of phospholipids within the membrane [5]. How a bacterial spore maintains such low cytoplasmic water content or water activity even when suspended in pure water is not yet understood [34].

32.5.6 Adaptation (Repair of Protein)

Bacterial membrane proteins are responsible for detecting environmental signals, promoting downstream regulatory events, and metabolic functions [18]. Protein could be damaged by aggregation, enzymatic inactivation, and changes in tertiary architecture [55, 56]. This damage can be linked to desiccation resistance and intracellular Mn/Fe concentration ratios [57]. Desiccation stress can enhance rapid synthesis of stress proteins. Heat shock proteins (HSP) have the ability to counterbalance the loss of water by the formation of hydrogen bonds to other molecules [58].

The efficiency of DNA repair and replication systems determines whether a bacterial cell lives or dies [59]. The protection theory against reactive oxygen species indicates that bacteria have robust systems of protecting proteins [57] and cells use DNA to stabilize proteins, thus it could be a molecular shield against desiccation stress [60].

32.5.7 Adaptation (Biofilms Formation)

Microbial biofilms provide better protection against various stresses than planktonic growing cells. These microbes are in strong isolation and embedded in a matrix of polysaccharides, proteins, lipids, and nucleic acid; and they possess the ability to cope in extreme temperature, hypersalinity, periodic desiccation, low temperature, disinfectants, and relative humidity

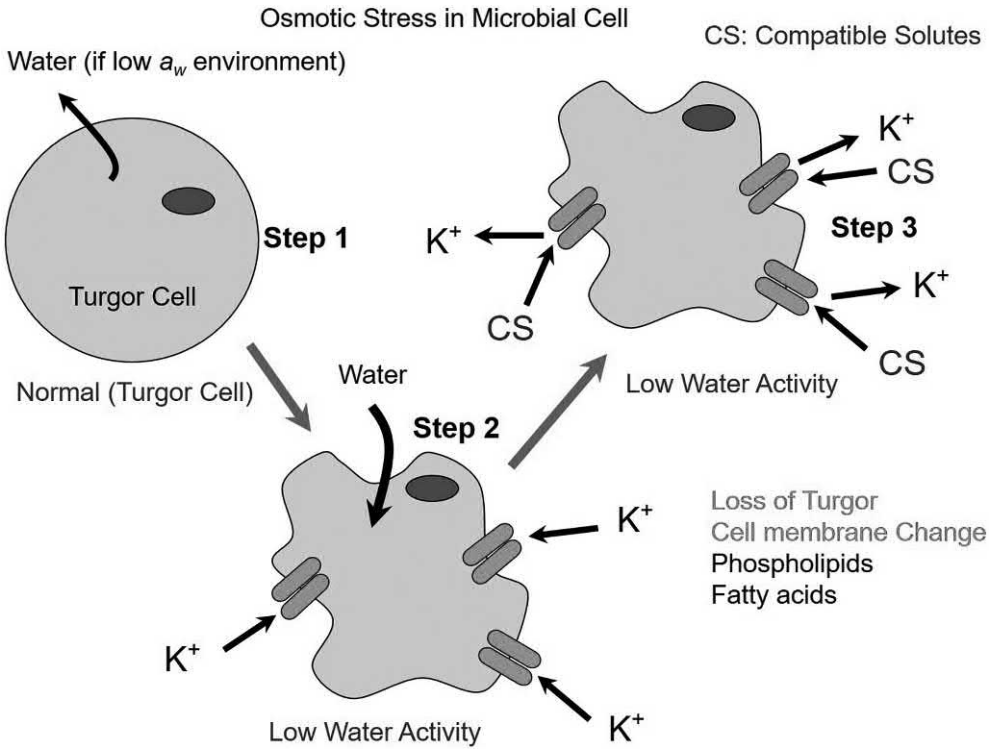

FIGURE 32.6 Microbial response to low water activity. (Adapted from Rahman [148].)

variations. The results affect maturation, physiology, antimicrobial resistance, virulence, and dispersal [61].

The desiccation tolerance is linked to the protective effect of the biofilm matrix due to the following reasons [18]: (i) aggregate formation and modifications in cell envelop composition and fluidity and/or shift in metabolism; (ii) formation of water-rich hydrated gel (about 95% moisture) around bacterial cells [62–64]; (iii) formation of upper (i.e., separation between cells) and lower layers, which are surrounding milieu [17, 65–67]; (iv) nutritional advantages due to concentrated organic molecules and ions close to the cells [68] and energy generation and cell envelop structure [69]; (v) high hydrophilic properties due to the formation of exopolysaccharides and extracellular DND and peptidoglycans [70, 71]; (vi) upregulation of alginate synthesis genes as well as flagella genes [72]; and (vii) formation of outer membrane antigen of lipopolysaccharide and cell surface components, like fimbriae and cellulose [73].

Salmonella can survive for extended periods of time in low-moisture environments. Finn et al. [74] isolated 46 *Salmonella* from low moisture survival phenotypes. Most of the isolates could form biofilms with different characteristics: (i) 57% being positive for curli fimbriae production and (ii) 75% was characterized with positive for cellulose production. These are linked with stronger biofilm formation. Cellulose-producing isolates were better survivors when exposed to a biocide compared with cellulose-negative isolates. Eighteen serotypes showed that glycerol was least inhibitory as compared to salts (i.e., sodium chloride and potassium chloride), and it was related to the biofilm.

32.5.8 Adaptation (Genetic)

The genetic components controlling osmoregulation in microorganisms are being investigated. *Escherichia coli* appears to have evolved a particularly advanced scheme for protection against osmotic stress through a proline-overproduced mutation, which confers osmotolerance. *Klebsiella pneumoniae* experiences an increase in intracellular free proline when it is exposed to high levels of sodium chloride. Thus, an enhanced level of osmoresistance in the organism results in its ability to fix nitrogen while under osmotic stress [5]. Considering *Salmonella*, a total of 266 genes were differentially expressed under desiccation stress as compared with a static broth culture [75]. It was observed that the osmoprotectant transporters *proP*, *proU*, and *osmU* (STM1491 to STM1494) were highly upregulated by drying, and deletion of any one of these transport systems resulted in a reduction in the long-term viability. The *proP* gene was found to be critical for survival; and *proP* deletion mutants could not survive long periods under desiccation (i.e., undetectable after 4 weeks) [76]. A total of 138 genes were differentially expressed, with upregulation observed for genes such as *proP*, *proU*, and the phosphate transport genes (*pstACS*) when rehydrated. Considering *Salmonella*, Chen et al. [77] identified the fatty acid biosynthesis associated genes in low water activity foods. They observed that low water activity increased expression of gene fabA (involved in unsaturated fatty acid biosynthesis), while the increased expression of cfa was associated with cyclopropane fatty acid synthesis. Salmonella ARI-33 always showed its potential in enhancing *Salmonella* survival as compared to other bacteria in low a_w foods.

32.6 FAT OXIDATION

Figure 32.2 shows a quality loss by oxidation can occur below monolayer value (i.e., line bn). If a food is susceptible to oxidation of unsaturated fats, such as occurs in cereal grains, the rate increases as water activity decreases below the monolayer. Oxidation and rancidity are aggravated by drying of foods to very low moisture levels [78]. Attack by oxygen is also responsible for pigment instability and loss of vitamins and sometimes initiates nonenzymatic browning reactions [79]. The attachment of an oxygen molecule to a binding site of a protein would produce an incongruity in the aqueous covering sheath, which could distribute the hydration structure of neighboring sites [80]. Competition with oxygen is not the sole basis for explaining the protective effects of water. The bond energy of the adsorbed water would inhibit interactions between polar groups on adjacent carbohydrate or protein molecules and thereby preserve rehydration ability, reconstitution ability, and texture of foods [78]. Moreover, with respect to fat oxidation, the catalytic effect of metallic compounds is reduced when they form coordination spheres with polar groups [81].

Water is important in lipid oxidation because it acts as a solvent, mobilizes reactants, and interacts chemically or by hydrogen bonding with other species. The basic protective function that water exhibited when the moisture content increased the absolute dry state could be accounted for by two factors: (i) water interacts with metal catalysts, making them less effective through changes in their coordination sphere; and (ii) water hydrogen bonds with hydroperoxides, tying them up so that these were no longer available for decomposition through initiation reactions. When the moisture content is higher than the value at the monolayer, the solvent and mobilization properties of water become more important and the catalysts present are more easily mobilized, and possible swelling of the solid matrix exposes new catalytic sites, making oxidation rates even higher [82, 83]. Thus foods having unsaturated fat should be kept at the critical water activity to maximize shelf life. Ling et al. [84] studied the lipid oxidation (hydrolytic rancidity, i.e., free fatty acid formation; and oxidative rancidity, i.e., peroxide value) in radio-frequency-heated bran as a function of water activity. The hydrolytic rancidity showed a sigmoidal shape similar to line abc in Figure 32.2 (i.e., rate of free fatty acid decreased with the decreasing water activity and reached the lowest value at water activity 0.241). This could be due to the low availability of water at low water activity, thus effectively inhibiting the hydrolysis of lipid [85]. In the case of oxidative rancidity, it showed a similar curve as nbc in Figure 32.2 (i.e., rate decreased with decreasing water activity and reached to the lowest value 0.111 meq/kg day at water activity 0.241 and then rapidly increased to 0.747 meq/kg day with a further decrease in water activity to 0.141). Bell [86] mentioned that the oxidation rate could increase as water activity decreased lower than its water activity corresponding to the monolayer of water. This could be due to direct contact of oxygen molecules and lipids, as a water protective layer does not exist. Similar minimum water activity values are reported as 0.33 for dried foods [87]; 0.23 to 0.43 for oatmeal muesli, peanuts, and pork scratchings [88]; and 0.44 for macadamia nut [89].

The water activity at the BET monolayer can be defined as the *critical water activity*. Autoxidation of lipids occurs rapidly at low water activity levels, decreasing until a water activity range of 0.3–0.5 is reached [90]. At low water content, especially in porous substrates in the complete absence of water, peroxidation of unsaturated lipids proceeds very rapidly. The addition of small quantities of water tends to produce a protective effect if the substrate is still free of oxidation products and reactive intermediates. However, reactions of oxidation products with proteins follow a more complex pattern [91]. In a model system consisting of methyl linoleate and lysozyme, the free radicals and other reactive species formed by the linoleate react with the protein, resulting in increased fluorescence, decreased enzyme activity, and decreased protein solubility. Water activity has an inhibitory effect on the initial oxidation of the lipid, but the secondary reactions of the lipid degradation products with the protein are accelerated by increasing water activity [92]. Schaich [93] studied free radical formation in proteins reacted with peroxide lipids and found that the amount and type of free radicals formed in the proteins were strongly affected by water activity. It appears that water facilitates the recombination of free radicals and as a consequence the steady-state concentration of radicals, whereas various radical-initiated processes such as protein cross-linking increase at high water content. In the case of freeze-dried model systems, certain amino acids including histidine, βamino-butyric acid, lysine, and cysteine showed substantial antioxidant activity [94].

32.7 NONENZYMATIC ACTIVITY

Browning reactions in foods affect the nutritional value as well as color and texture [95]. The induction period, defined as the time to visually detectable browning, is inversely proportional to water activity [96, 97]. Browning reactions are influenced by the types of reactant sugars and amines, pH, temperature, water activity, and the types of solutes or humectants used to adjust the water activity [90].

32.7.1 Types of Browning

There are three major pathways by which nonenzymatic browning can occur: high-temperature caramelization, ascorbic acid oxidation, and the Maillard reaction [95]. The browning reactions of sugars heated above their melting point in the absence of proteins or amino acids are called caramelization. This can be either beneficial or detrimental to the quality of a food product and can be prevented by avoiding high-temperature processing and low storage temperatures. It is enhanced in alkaline or acid conditions and is used to make commercial caramel colorings and flavors.

Ascorbic acid (vitamin C) oxidation, a second type of browning reaction, is catalyzed by low pH and elevated temperatures. The decomposition products resulting from the oxidation of ascorbic acid cause a brown discoloration as well as

decreased nutritional value. The Maillard reaction is a result of reducing compounds, primarily sugars, reacting with proteins or free amine groups. This changes both the chemical and the physiological properties of the protein. In general, the accumulation of brown pigments is the most obvious indication that Maillard browning has occurred in a food containing both carbohydrate and protein. It is used as an indicator of excessive thermal processing in the milk industry [95]. In the early stages of the Maillard reaction, the carbonyl group of the reducing sugar reacts with the free amino group of the amino acid to form a Schiff base and then the N-substituted glycosylamine as well as a molecule of water. Glycosylamines are converted to the 1-amino-1-deoxy-2-ketose by Amadori rearrangement (cyclization and isomerization) [98]. The Maillard reactions forming Amadori compounds do not cause browning but do reduce the nutritive value [99]. The advanced Maillard reaction has five pathways. The first two pathways start from the 1,2-enol or 2,3-enol forms of the Amadori product, yielding various flavor compounds. The third pathway is Strecker degradation, which involves oxidative degradation of amino acids by the dicarbonyls produced in the first two pathways. The fourth pathway involves transamination of the Schiff base. The fifth pathway starts with a second substitution of the amino-doxy-ketose. The final step of the advanced Maillard reaction is the formation of many heterocyclic compounds, such as pyrazines and pyrroles [98]. Brown melanoidin pigments are produced in the final stage of the Maillard reaction. The pigments are formed by polymerization of the reactive compounds produced during the advanced Maillard reaction, such as unsaturated carbonyl compounds and furfural. The polymers have a molecular weight greater than 1000 and are relatively inert [99]. These pathways depend upon environmental conditions such as pH and temperature.

32.7.2 Factors Affecting Browning

The browning reaction rate increases from water activity at the BET monolayer, sharply increases to a maximum, and then decreases (Figure 32.2). Water can retard the rate of the initial glycosylamine reaction of which water is a product. This results in product inhibition by some of the intermediate reactions. A second factor is the dilution of reactive components with increasing water content. The mobility of the reactive species increases due to a decrease in viscosity with increasing water activity. However, the reactive species increase due to a decrease in viscosity with increasing water activity. However, the first two factors eventually overcompensate for the decreased viscosity at higher water activity, and thus the overall rate of browning decreases [95].

Wolf et al. [100] demonstrated that losses of free lysine and methionine were highly dependent on water activity, protein, and sugar. Thermal degradation of both amino acids followed first-order kinetics, and rates decreased at 65°C and 115°C with increasing water activity. A more rapid decrease of lysine, tryptophan, and threonine at higher water activity in model systems is observed when heated at 95°C [101]. The retention of tryptophan was greater than lysine at water activity 0.75, but lysine retention was greater than that of tryptophan at water activity 0.22. At higher water activity, the Maillard reaction predominates and a rapid loss of lysine occurs. At lower water activity, browning proceeds at a slower rate and reactions involving the indole ring of tryptophan become significant [98]. Glucose utilization in a model system consisting of glucose, monosodium glutamate, corn starch, and lipids during nonenzymatic browning was investigated by Kamman and Labuza [102]. The rates of glucose utilization at water activity 0.81 were higher than at 0.41. Lipids accelerated the reaction rates at 0.41 but had virtually no effect at 0.81 water activity. Liquid oil is more effective than shortening in increasing the degradation rate of glucose. These can be explained by the mobility of solutes in both water and oil [98].

Cerrutti et al. [103] studied browning in a model system consisting of lysine, glucose, sodium chloride, and phosphate buffer. They showed that water had little or no effect on the rate of glucose loss at water activity 0.90–0.95, but the rate was highly dependent on temperature and pH. Similar behavior was observed for the accumulation of 5-hydroxymethylfurfural, fluorescent compounds, and brown pigments. Seow and Cheah [104] found that nonenzymatic browning decreased with an increase of water activity and temperature in a water–glycerol–sorbate–glycine model system at pH 4. For dehydrated orange juice (a_w: 0.44) stored at 30°C and 50°C, the total amino acids lost due to nonenzymatic browning were 30% and 65% of initial concentration [105].

32.7.3 Maximum Browning Region

The region where the maximum browning occurs is usually near 0.65–0.80 water activity. In model freeze-dried foods the maximum browning rate is in the range of 0.40–0.67 [106], in whey powders at 0.44 [95], and in dehydrated foods in the range of 0.65–0.75 [107]. Petriella et al. [108] found that water had relatively less effect on the browning rates at water activity of 0.90–0.95. At this range, pH and temperature are the determining factors. At very high water content, i.e., water activity greater than 0.95, moisture strongly inhibits the browning rate by diluting the reactive species [90]. Warmbier et al. [107] studied the influence of solutes on the maximal range of browning. For example, if glycerol is employed to reduce water activity, the range of maximal browning shifts from 0.65–0.75 to 0.40–0.50. They concluded that glycerol can influence the rate of browning at lower water activity values by acting as an aqueous solvent and thereby allowing reactant mobility at much lower moisture values than would be expected for water alone. The overall effect of glycerol or other liquid humectants on the maximum for nonenzymatic browning is to shift it to a lower water activity [95]. Obanu et al. [109], on the other hand, observing browning in glycerol-amino acid mixtures stored at 65°C, concluded that glycerol itself might participate in the browning reaction. Moreover, Troller [90] indicated that product quality relative to browning could be improved by reducing the water activity and, more importantly, the temperature during the final stage of drying. It is somewhat paradoxical that at water activity

levels that minimize browning, autooxidation of lipids is maximized.

32.8 ENZYMATIC ACTIVITY

Enzyme-catalyzed reactions can proceed in foods with relatively low water contents. Karel [91] summarized two features of results mentioned in the literature as follows: (i) the rate of hydrolysis increases with increasing water activity, with the reaction being extremely slow at very low activities; and (ii) at each water activity, a maximum extent of hydrolysis appeared, which also increases with water content. The apparent cessation of the reaction at low moisture cannot be irreversible in activation of the enzyme, because upon humidification to a higher water activity, hydrolysis is resumed at a rate characteristic of the newly obtained water activity [91]. Silver [110] investigated a model system consisting of avicel, sucrose, and invertase, and found that the reaction velocity increased with water activity. Complete conversion of the substrate was observed for water activities greater than or equal to 0.75. Below water activities of 0.75, the reaction continued to 100% hydrolysis. In solid media, water activity can affect reactions in two ways: lack of reactant mobility and alteration of active conformation of substrate and enzymatic protein [111]. Effects of varying the enzyme-to-substrate ratios on reaction velocity and the effect of water activity on the activation energy for the reaction could not be explained by a simple diffusion model, but required more complex postulates [91]: (i) the diffusion resistance is localized in a shell adjacent to the enzymes, and (ii) at low water activities, the reduced hydration produces conformational changes in the enzyme affecting its catalytic activity. Tome et al. [111] tested the simple diffusion-related hypothesis on the basis of experiments in liquid systems in which water activity was reduced by the addition of glycerol, ethylene glycol, propylene glycol, diethylene glycol, sorbitol, methanol, or ethanol. In these solutions the effects of polyphenoloxidase on tyrosine were very similar to those obtained in solids systems.

The optimum pH of activity is shifted slightly toward alkaline values. Three characteristics curves were observed: (i) for low water activity there was an almost total inhibition; (ii) in the intermediate range, the reaction rate was very dependent on water activity; and (iii) for high water activity zones, activity was weakly affected by organic additives. In general, the rate increased rapidly with increasing water activity, and the reaction stopped at a certain level before all reactants were consumed; the higher the water content, the higher was the plateau. The authors were unable to find a correlation of enzyme activity with viscosity, solubility of oxygen and tyrosine, or dielectric constant. It also appeared that the more the mixture deviated from the ideal, the more the enzymatic activity was inhibited, regardless of whether the deviation was positive or negative. Thus, solvent–water interaction is the main parameter in polyphenoloxidase inhibition. The minimum water activities for enzymatic reactions in selected food systems are given in Table 32.8.

32.9 VITAMIN LOSS

The nutrition loss of dehydrated foods depends on the storage temperature, light, oxygen, and water activity. The loss of thiamine due to heating is affected by (i) the state of the thiamin molecule (incorporated into enzyme or protein-bound), (ii) pH (the rate of destruction increases especially in the alkaline region), (iii) metals (free metals act as catalysts to increase the rate of thiamine destruction), and (iv) oxygen (oxygen can accelerate thiamine destruction especially in solutions above 70°C) [112]. The thiamin destruction during heat treatment strongly depended on pH and insignificant influence of water activity within 0.9–1.0 values [113]. Products with a pH value of 3 showed excellent thiamin retention, while those with a pH value approaching 7 showed a strong instability during the thermal process. The destruction of thiamin in the model system was less than 5% at storage temperatures ≤37°C and was independent of water activity at a_w ≤0.65. A significant increase in the thiamin loss occurred in the model system stored at 45°C when the water activity was at or above 0.24. Riboflavin is considered more heat stable than thiamin but highly sensitive to degradation by light. The stability of riboflavin in dry products is considered to be excellent in the absence of light [114]. With only one expectation, the reaction rates of vitamins A, B_1, B_2, and C increased with increasing water activity 0.24–0.65 [98]. B vitamins are more stable than vitamins A and C at various water activity values [98].

Hemery et al. [115] observed that the type of packaging (i.e., paper, PET, and aluminum foil), fortification (i.e., ferrous sulfate), water activity (i.e., 0.65 and 0.85), and storage temperature (25°C and 40°C) affected the vitamin A stability in fortified wheat flour. Considering aluminum foil (i.e., where there was less change in water activity during storage), both storage time and temperature severely affected the stability, whereas it was marginally affected by the addition of ferrous sulfate and water activity. For example, in the case of water activity 0.65 stored at 1.5 months of storage, retentions were 80% and 65% when storage temperature was 25°C and 40°C, respectively. Similarly, in the case of water activity 0.85, the retentions were 85% and 60% when stored at 25°C and 40°C, respectively. After 3 months storage, the retentions were nearly 55% (water activity 0.65) to 50% (water activity 0.85), and 15% (water activity: 0.65 and 0.85) when stored at 25°C and 40°C, respectively. There was little difference between 3 and 6 months of storage. In addition, vitamin A retention was related to the extent of oxidation reactions that occurred in flours during storage.

32.10 TEXTURE

Rockland [116] defined food texture as a function of localized moisture sorption isotherms as follows: (i) region I (low water activity)—dry, hard, crisp, and shrunken; (ii) region II (intermediate water activity)—dry, firm, and flexible); and (iii) region III (high water activity)—moist, juicy, soft, flaccid, swollen, and sticky). Table 32.9 shows textural characteristics of model food products via water activity. The effect

TABLE 32.9
Critical Values for Ingredients in Model Food Products

Moistness	Crispness	Chewiness	Toughness
Cereal		>0.40	<0.50
Fruit	<0.30	>0.50	>0.30
Nuts		>0.65	

Source: Bourne [117].

of water activity on textural measurements for different types of foods was reviewed by Bourne [117]. At this time there are insufficient data to predict what the textural properties of a given type of food will be at a given water activity, and no sound theories exist to predict in advance the textural properties of a food at a given water activity. Cenkowski et al. [118] studied the mechanical behavior of canola kernels by bringing them to equilibrium, adsorption or desorption, at the same final moisture. The ratio of elasticity was 18–38% higher for kernels brought to equilibrium through adsorption than those through desorption for a moisture range of 9.5–7.5% (dry basis). At higher moisture contents, the differences in module of elasticity were not significant. For dry snacks, the loss of crispiness occurred close to the BET monolayer [88]. In the case of potato chips [119] and corn chips [120] the critical water activity when the product was unacceptable was found at 0.40 water activity. The change in sensory crispiness of potato chips, popcorn, puffed corn curls, and saltines generally fell in the 0.35–0.50 water activity range [121]. Instron analysis showed that the force–deformation curve changed distinctly near critical a_w for saltines and puffed corn curls, while the curve changed more gradually with increasing a_w for popcorn.

32.11 ISSUES OF WATER ACTIVITY CONCEPT

The major drawbacks of the water activity concept are the validity of equilibrium conditions, discontinuity or break in the isotherm, effects of different solutes, dilution effects, and it explains only the binding nature of water without giving an explanation of molecular mobility.

32.11.1 Validity of Equilibrium Conditions

Water activity is defined at equilibrium, whereas foods with low and intermediate water content may not be in a state of equilibrium. Instead, they may be in an amorphous multistate, which is very sensitive to changes in moisture content and time. In low-moisture and intermediate-moisture foods, the concept of water activity may be meaningless because the measured vapor pressure of water is no longer the equilibrium vapor pressure as defined in the literature. A stationary state may be reached under a given set of environmental conditions and mistaken for equilibrium. In moisture-sorption studies, the situation is further complicated if the amorphous material undergoes a glass–rubber transition during the course of the measurement.

Chirife and Buera [7] believe that an analysis of various literature data may throw some light on equilibrium aspects. An important comprehensive collaborative study within the framework of the European Corporation in the Field of Scientific and Technical Research (COST) was conducted to determine the precision of data (e.g., repeatability or reproducibility) in the determination of sorption isotherms. In the water activity range of interest to microbial growth (0.6–0.9), the average standard deviation of all data from 24 laboratories was ±2.6% for equilibrium moisture content of microcrystalline cellulose (MCC) and ±3.8% for potato starch. The repeatability was 2% for both MCC and potato starch. Chirife and Buera [7] also reported data on isotherms from different sources of the same material and found good reproducibility within a wide range of water activity. Lomauro et al. [122] concluded from a study of a large number of foods that a pseudoequilibrium was reached when the moisture content (dry basis) did not change by more than ±0.5% during three consecutive sample periods at intervals of no more than 7 days. This criterion for equilibrium moisture content was compared with the values obtained after 6 months of storage in closed mason jars, which were considered to be very close to the equilibrium moisture content.

Lomauro et al. [122] concluded that the foods tested reached (or were very close to) equilibrium within 1 month, based on the above criterion. Various authors reported their equilibrium times for isotherm determinations of different food systems using the gravimetric static method over saturated salt solutions, and their equilibrium times in the gravimetric static method over saturated salt solutions, and it was observed that equilibrium times ranged mostly between 1 and 4 weeks, depending on the temperature and relative humidity. Bizot et al. [123] utilized a practical equilibrium time of about 7 days (±0.02% water per 24 h) for a 1 g sample, but they also stored their starch samples over saturated salt solutions for 2 years. They noted a slow drift in desorption pseudoequilibrium, but there was only a 1% difference in water content (dry basis) over this long time. Thus water activities measured are likely to be close to equilibrium, and the differences should be within the uncertainties associated with the experimental determination of isotherm [7].

32.11.2 Break in the Isotherm

Chirife and Buera [7] reviewed sorption isotherms of fruits containing sugars (which have ability form crystals) that constitute a nonequilibrium system. For example, in raisins the discontinuities in the isotherm are not noticed at water activity 0.30–0.90, suggesting that sugars remained amorphous even at very high temperatures above the glass transition. The sorption isotherms of other fruits reported in the literature did not show discontinuities [7]. Bolin [124] observed little effect on isotherm with raisins sealed in glass jars held at 21°C or 32°C for up to 12 months. This suggested that nonequilibrium effects are very slow, at least in their

experiments. Chirife and Buera [7] also presented data on fruits, but they overlooked the crystallization of sugars in dairy products and formulated products having sugars or salts, as discussed in Chuy and Labuza [125] and Saltmarch and Labuza [126]. A recently developed dynamic sorption apparatus could measure the water activity within a couple of hours of sample size in the range of micrograms. Although the break in the isotherm creates a problem for the measurement, it provides a practical importance as far as detecting any change in structural components when stored on a specific water activity environment.

32.11.3 Effects of Solute Types

The microbial response may differ at a particular water activity when the latter is obtained with different solutes [7, 127]. Thus the proposed basis of water activity limit for growth may not be universal. Corry [128] reported that the survival of vegetative bacteria is influenced by nutrients in the food matrix. These influences show no consistent inhibitory pattern and are greatly affected by the matrix. Mugnier and Jung [129] studied the survival of bacteria and fungi in biopolymer gels with and without nutritive solutes. They observed that survival is increased at the point of mobilization of solute in the case of mannitol. While comparing a gram-positive bacterium and a gram-negative one, they concluded that low molecular weight compounds (C_3–C_5) had a deleterious effect on survival compared to higher molecular weight compounds (C_6–C_{12}), which had a protecting effect.

The glass–rubber state of solutes may also play an important role since higher molecular weight solutes have higher glass transition temperatures than low molecular weight solutes. The degree of protecting effect was in the order of mannitol > dextrin > ribose > glycerol. Above a certain amount of hydration (the mobilization point), there exists a second fraction of solute in the polymer system, which can serve as a true solvent for the microbial nutrient to maintain the organism's metabolic activity [9]. Brown [130] stated that freeze-drying of microorganisms with a nonelectrolyte such as glycerol or sugar reduces mortality during dehydration, storage, and rehydration. This indicates that the nonelectrolyte functions directly as a solvent molecule for nutrients. Gould [34] acknowledged that in some instances solute effects may depend on the ability of the solute to permeate the cell membrane. Glycerol, for example, readily permeates the membrane of many bacteria and so does not initiate the same osmoregulatory response as nonpermeate solutes like sodium chloride and sucrose, therefore there is a different, usually lower, inhibitory water activity. Scott [131] noted that minimal water activity for the growth of microbial organisms was independent of the solutes employed to adjust the water activity of a medium. It was observed later that some solutes were more inhibiting than others, thus water activity of a medium is not the only determining factor regulating microbial response. The nature of the solute used also plays an important role [132, 133]. This is referred to as *specific solute effects* by Chirife [134].

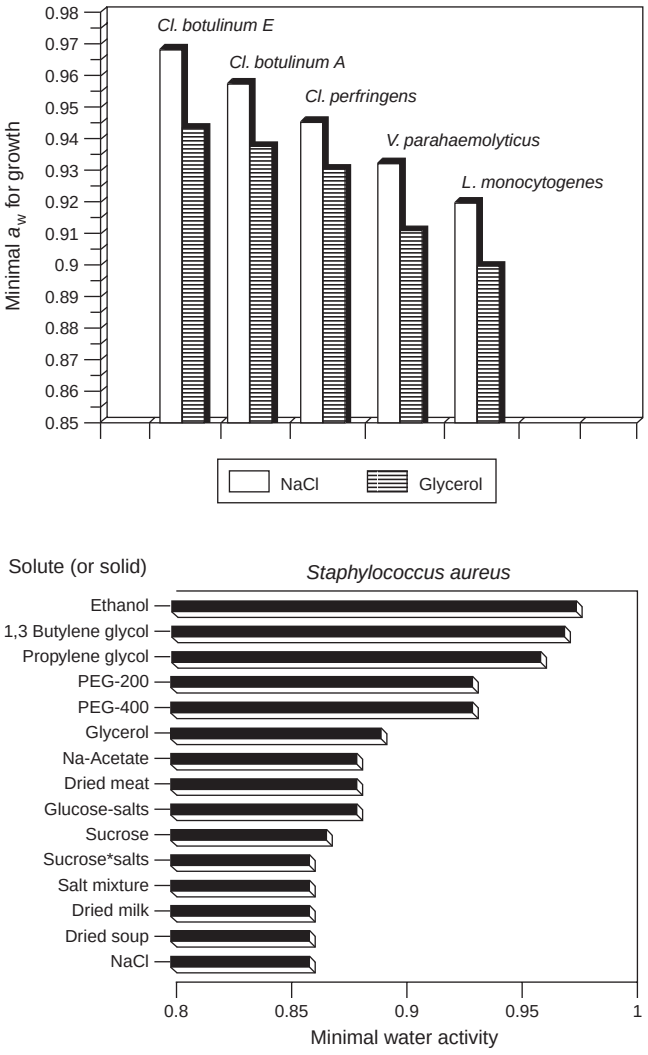

FIGURE 32.7 Effect of minimum water activity levels on growth of bacteria in different solutes. (From Chirife and Buera [7].)

Figures 32.7 and 32.8 compare the minimal water activity supporting the growth of various pathogenic bacteria when sodium chloride or glycerol are used to control the water activity. In all cases, glycerol is less inhibitory than sodium chloride. Glycerol readily permeates the membrane of bacteria and does not initiate the same osmoregulatory response as the nonpermeate solute sodium chloride [134]. It is conclusive that the water activity limit varies with the type of solute used and the microorganisms in the medium. Thus, it is important to identify the range of variation and the possible causes of variation. Table 32.4 shows the effects of solute types on *Staphylococcus aureus*. The range of water activity varies from 0.860 to 0.966. Propylene glycol is more effective in *S. aureus*, which is explained by Chirife [134]. The points in Figure 32.7 are distributed on both sides of the line, indicating a high correlation of water activity with growth and fluctuation due to other factors in the microorganisms. The effect of solute type on different microorganisms should be clearly identified in order to recognize generalized trends or at least the limitation of validity. Electron micrographs of *S.*

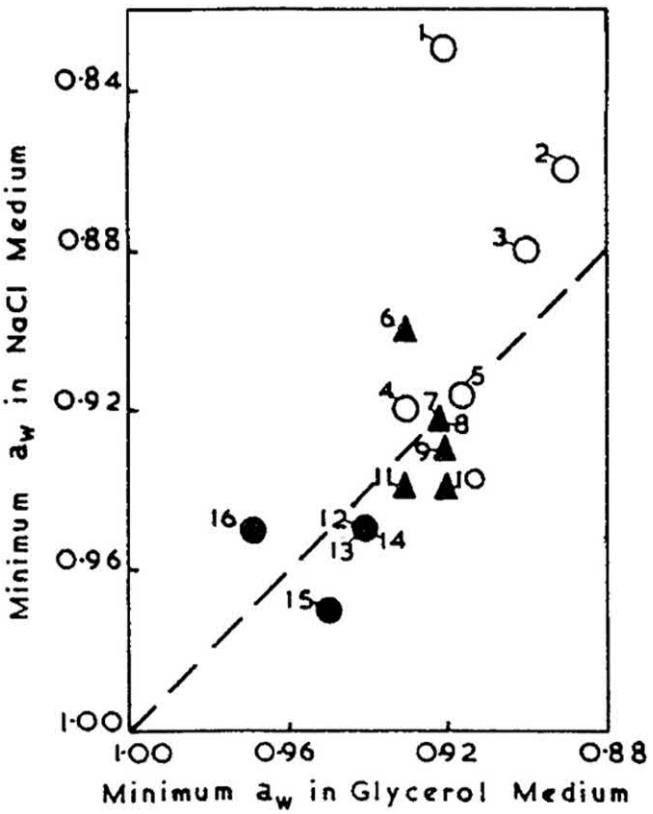

FIGURE 32.8 Relationship between the minimum water activity levels for growth of 16 species of bacteria in NaCl and in glycerol-adjusted medium. (From Christian [132].)

aureus after growing in a medium containing different solutes were analyzed [134]. Microbial cells subjected to sodium chloride and sucrose (a_w = 0.85) did not show any important morphological changes in the cells, thus the inhibitory effects of sucrose and sodium chloride against cells were primarily related to their ability to lower water activity, specific solute effects are not significant. In solutions of propylene glycol (a_w = 0.92), 1,4-butylene glycol (a_w = 0.85), and polyethylene glycol 400 and 1000 (a_w = 0.85) showed that these solutes caused dramatic morphological modifications in the cells. These antibacterial effects may be attributed mainly to the effects of these molecules on the membrane enzymes responsible for peptidoglycan synthesis.

Anand and Brown [135] observed that polyethylene glycol was more inhibitory to yeast growth than were glucose and sucrose at a similar water activity. Marshall et al. [136] evaluated the inhibitory effects of sodium chloride and glycerol at the same water activity on 16 species of bacteria. They found that glycerol was more inhibitory than sodium chloride to relatively salt-tolerant bacteria and less inhibitory than sodium chloride to salt-sensitive species. Lenovich et al. [9] showed that the type of solute influences resistance to sorbate in *Saccharomyces rouxii*, thus indicating an interactive effect of solute type and preservative. Buchanan and Bagi [137] studied the effects of solutes (mannitol, sorbitol, and sucrose) in combination with four pH levels and three incubation temperatures on the growth of *Escherichia coli*. In addition to water activity, the growth kinetics were influenced by temperature and pH, and inhibition order followed as sorbitol > mannitol > sucrose. In addition, at higher water activity levels, particularly when temperature and pH were nonlimiting, the differences between the humectants were minimal. However, as the environment was made more inhospitable, differences due to humectants were observed. Effects of solutes were insensitive to the *Lactobacillus casei* when sodium chloride or sorbitol was used to control the water activity [138]. The time of germination of spores appeared to vary depending on the water activity and solute type during spore production [124]. Resistance or susceptibility of microorganisms to antibiotics depends on the water activity and types of solutes. For example, resistance of *Streptococcus thermophilus* increased when water activity was lowered with glycerol, whereas susceptibility increased when water activity was adjusted with glucose and acid production was higher when sucrose was used. Susceptibility to gentamycin increased in both species (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) with reduced water activity [139]. Although there are differences in microbial stability when different solutes are used to achieve the same water activity, that does not mean that the concept of water activity is invalid [140]. It has been shown repeatedly in the literature that each microorganism has a critical water activity below which growth cannot occur. For example, pathogenic bacteria cannot grow below a water activity of 0.85–0.86; yeasts and molds are more tolerant of reduced water activity, but usually no growth exists below a water activity of about 0.6 (Table 32.6).

32.11.4 Molecular Mobility and Glass Transition

The water activity concept is mainly based on the binding nature of water and its relationships with the food stability or reactivity. However, molecular and/or matrix mobility also play a significant role in determining food stability, which could not be explained by water activity. The glass transition concept could explain more regarding the molecular or matrix mobility. Recently, Rahman [141–143] reviewed the applications of the glass transition concept in food product stability during storage. Slade and Levine [144] and Franks [145] proposed that water activity can serve as a useful but not the sole indicator of microbial safety. Slade and Levine's [144] hypothesis stated that water dynamics or glass–rubber transition may be used instead of water activity to predict microbial stability.

Slade and Levine [146] also reported that for matched pairs of fructose and glucose at equal solute concentrations, fructose produced a much less stable system in which mold spores germinated much faster, i.e., the solute with lower ratio of melting temperature to glass transition temperature ($\alpha = T_m/T_g$) allowed faster germination. Thus, the following order of antimicrobial stabilization was predicted by Slade and Levine [146]: glycerol (α = 1.62) > glucose (α = 1.42) > mannose (α = 1.36) > fructose (α = 1.06) and sucrose (α = 1.43) > maltose (α = 1.27). The germination of *Aspergillus flavus*, *Aspergillus niger*, and *Eurotium herbariorum* did not follow this sequence

[147]. For example, germination times for all three molds in fructose or glucose were always greater than in glycerol. In all cases, germination time increased when the water activity decreased; the relative effect, however, depended both on the solute type and the mold. None of the molds studied germinated in solution of propylene glycol (at a_w = 0.85 or 0.90; α = 1.27) after 70 days of incubation at 28°C [147]. This is simply because the behavior of propylene glycol cannot be explained on the basis of mobility or water activity effects alone, since this molecule possesses specific antimicrobial effects already recognized in the literature [147]. However, further studies from the microbiology groups could clarify the aforementioned results.

Chirife and Buera [7] also showed evidence that above glass transition, microbial growth is inhibited in prunes, and that below glass transition, microbial growth is possible in wheat flour. Overall, both the water activity and glass transition concepts are not valid in all systems and conditions. Recent trends are to apply multiple concepts, such as water activity, glass transition, and pH together in order to determine the chemical, physical, and microbiological changes in foods. However, a more unified approach including water activity, pH, glass transition, and preservatives needs to be developed. More discussions on glass transition concept and other factors are provided in the chapters on glass transition and state diagram (Chapters 23 and 24, respectively).

32.12 CONCLUSION

Food stability in terms of chemical reaction and microbial growth in foods is very complex due to its multiple factors and their interactions. Water activity could be the first step in determining food stability as different critical limits are developed. However, these critical limits and relationships shifted as other hurdles or factors are involved. This does not invalidate the concept but rather reduces its universality. Therefore, it is necessary to develop and progress a multiconcept theoretical framework, especially linking water activity, molecular mobility, pH, and storage temperature.

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33 Surface Treatments and Edible Coatings in Food Preservation

Elizabeth A. Baldwin

CONTENTS

33.1 INTRODUCTION

Consumer interest in health, nutrition, and food safety combined with environmental concerns have renewed efforts in edible coating research. Renewable and abundant resources are available for use as film-forming agents that could potentially reduce the need for synthetic packaging films that add to waste-disposal problems. Alternatives to petroleum-based packaging include naturally occurring lipid, resin, protein, and carbohydrate film formers and their derivatives. In fact, coating techniques had been in use for centuries, before the development of plastic polymers. For example, beeswax was used to coat citrus fruit to retard water loss in China during the 12th and 13th centuries [1], and "larding" (coating food with fat) to prevent desiccation was practiced in 16th-century England [2]. The use of synthetic and natural waxes and resins to coat fresh fruits and vegetables has been researched and practiced in the United States, the United Kingdom, and Australia since the 1930s [3–9]. Development of edible coatings for use on meat products was first reported in the late 1950s [10–14].

Currently, edible coatings and films are commonly used on many commodities, such as candies, fresh fruits and vegetables, and processed meats. New research seeks to expand and improve coating technologies and materials to enhance food stability and quality. Other surface treatments for foods include application of antioxidants, acidulants (or other pH-control agents), fungicides, preservatives, and mineral salts, some of which are more extensively covered in other chapters of this volume.

33.2 RATIONAL FOR USING EDIBLE COATING AND SURFACE TREATMENTS

Edible coatings serve many purposes in food systems. Coatings are used to improve appearance or texture and reduce water loss. Examples include the "waxing" of apples and oranges to add gloss and reduce shrinkage due to water loss or the coating of candies to reduce stickiness [15, 16]. Use of antioxidants and sulfites to preserve fresh appearance in minimally processed fruits and vegetables or processed foods is well-reviewed by Sapers [17] and Sherwin [18]. Antioxidants are used to reduce browning of cut apple and pear [19–22], potato [23, 24], mushrooms [25, 26], and shellfish [17, 27, 28], and to preserve the color of fish [29], which is more extensively reviewed in another chapter. Fungicides are used to reduce decay of whole fruits [30]; and salts, such as calcium, are reported to delay ripening, increase firmness, improve appearance, and enhance the disease resistance of fruits [31–35]. Preservatives, acidulants, and to some extent antioxidants and sulfites reduce surface microbial populations on fresh-cut produce and processed foods [36, 37]. Many of these treatments are covered in more detail in other chapters.

This review concentrates on the use of edible coatings alone and as carriers of antioxidants [38–41], preservatives [41–46], acidulants [40, 41, 47], salts [34], and fungicides [48–55]. Coatings have been shown to increase the efficiency of preservatives [41, 44], but fungicides sometimes have reduced activity in a coating system [51, 52]. Edible coatings can also encapsulate flavor for preservation, storage, or controlled release in food systems [56]. Another advantage is the retention of flavor volatiles in coated fruit [57]. Coatings and films can slow deteriorative changes in coated products by reducing desiccation and oxidation and, in some cases, by creating a modified atmosphere around coated products [15, 16]. Coatings can reduce the migration of lipids (fats and oils) in confectionary products [58] and fried foods [16, 58, 59], or separate components of different water activity [60, 61]. Formulations can be designed to carry desired additives (including antioxidants, acidulants, chelators, preservatives, and fungicides) that help to extend product stability and, therefore, shelf life [62]. Edible films and coatings can also improve mechanical handling properties and structural integrity of various food products by helping fruit slip over packing lines with less injury [63] or by holding toppings in place during product distribution [64].

33.3 MECHANISM OF ACTION

33.3.1 Permeability Properties of Coatings

Permeability of films and coatings to water vapor, gas, solute, or lipids is an important property to consider when selecting film materials or for tailoring coatings for specific commodities. Permeability of a barrier is calculated from a combination of Fick's first law of diffusion and Henry's law of solubility. This is used to determine flux of permeate through a nonporous barrier, assuming that the barrier has no imperfections. Permeance is a measure of flux without accounting for barrier thickness and used for performance evaluation of a film rather than describing its property. Transmission rate describes permeance without accounting for film thickness or the partial pressure gradient of permeate. Resistance describes the ability of a material to serve as a barrier to the diffusion of permeate. A detailed discussion of terms, equations, and theories of permeability are presented by Donhowe and Fennema [64] and McHugh and Krochta [65].

Permeability properties of edible films are often unpredictable due to the absence of a homogeneous structure and the often hydrophilic nature of most formulations [65]. The chemical composition and structure of the film-forming polymer affects film permeability in general. Highly polar materials with a high degree of hydrogen bonding exhibit low gas permeability, especially under conditions of low humidity, but are poor barriers to moisture. Nonpolar materials, such as lipids, provide good moisture barriers but are permeable to gases such as oxygen. The type of functional group on a polymer can also have an effect, depending on the resulting chain interaction, motion, and functional group (i.e., hydrophilic or hydrophobic). Ionic functional groups create strong polymer chain interactions, which restrict chain motion. This usually results in good oxygen barriers, but also hydrogen bonding with water and subsequent water absorption at high relative humidities (RHs), which, in turn, results in high rates of water vapor permeation. In addition, absorption of water disrupts intermolecular chain interaction, which increases permeability in general. This is the reason that films are often more permeable at high RHs [66]. Nonpolar groups result in a much less effective oxygen barrier film when present as the side chain but improve water permeability slightly.

Addition of low molecular weight components, or plasticizers, can affect film permeability and flexibility, often increasing both (especially water vapor permeability) by disruption of polymer chain hydrogen bonding [65, 67]. These components are generally added to decrease film brittleness by increasing elasticity/flexibility, resulting in less cracking and flaking of coatings.

The structure of the film-forming polymer is also important in terms of influencing the permeability properties of a film. Polymer chain packing, whether it is tight or loose due to bulky side chains, results in increased or decreased permeability properties, respectively [65]. The molecular weight and crystalline structure of a polymer can have an effect [67]. Lipids can exist in different crystalline states, which result in different barrier properties, with a higher degree of crystallinity resulting in lower permeability. Temperature affects polymer mobility [68] and thus permeability. Higher temperatures (above the "glass" transition state) result in polymers that are more mobile (plastic amorphous state) and have relatively increased permeability properties compared to when they exist as "glasses" or in brittle form at lower temperatures [65]. Even without going through a structural transition, oxygen transmission through protein films was affected by temperature [68]. Orientation of polymers to the flow of permeate can affect permeability properties. For example, the packed arrangement of wax crystals perpendicular to the direction of gas flow presents a better barrier [69] than when parallel to the direction of flow.

Cross-linking of polymer chains with ions or enzymes can lower permeability values as well as change the pH (depending on the isoelectric point in the case of protein films) [65, 70]. The addition of hydrophobic materials (lipids) to a hydrophilic film former to make a composite coating can sometimes improve the moisture-barrier properties of the hydrophilic film former. This was demonstrated for a matrix of methylcellulose and hydroxypropyl methylcellulose combined with saturated C_{16} and C_{18} fatty acids laminated with beeswax and with a chitosan film containing lauric acid [71–73]. This can also be achieved by forming bilayer films from hydrophilic and hydrophobic materials. An example of this was reported for hydroxypropyl methylcellulose and a blend of stearic and palmitic acids [60, 74].

33.3.1.1 Effect on Water Loss

Water loss usually occurs in the vapor phase. Water vapor permeability describes the movement of water vapor through a film or coating per unit area and thickness, and determines the vapor pressure difference across the film at a specific temperature and humidity [75]. If pores, cracks, or pinholes form on the film surface, then water vapor flows through these areas directly, which is different from the dissolving and diffusion of water vapor through a film barrier [65]. Water vapor transfer through films is dependent on environmental conditions such as temperature and humidity, and thus should be tested under the conditions expected to be encountered by a specific product. Generally, the more hydrophilic the film-forming material, the more permeable the film will be to water vapor.

33.3.1.2 Effect on Gas Exchange of Fresh Fruits and Vegetables

33.3.1.2.1 Creation of a Modified Atmosphere for Coated Fresh Produce and Effect on Ripening

Cells of plant tissues, such as harvested fruits and vegetables, are physiologically active in that they consume oxygen (O_2) and produce carbon dioxide (CO_2) as they respire. When fruits or vegetables are sealed in semipermeable plastic packaging or coating, a modified atmosphere (MA) is created within the packaging or in the internal atmosphere of the fruit, in the case of edible coatings-depending on the permeability of the film or coating. During storage, fruit respiration continues to consume O_2 and release CO_2 [15]. If O_2 levels fall too low (below 1–3%, depending on the produce

and storage temperature), anaerobic reactions can occur, which result in off-flavors, abnormal ripening, and spoilage [76, 77]. Climacteric-type fruits are often harvested immature and ripen off the mother plant with an accelerated respiration pattern and ethylene production [76]. The high rates of respiration and ethylene production, which turn on genes regulating ripening and senescence, contribute to a relatively short shelf life for this type of produce. Ethylene production, like respiration, is a process that requires O_2. Low O_2 (below 8%) and high CO_2 (above 5%) concentrations slow respiration and retard ethylene production and, therefore, ripening [77]. High storage temperatures increase fruit or vegetable respiration [76] and exacerbate the effect of a coating or other packaging on the internal atmosphere of the coated produce. Low temperature, on the other hand, slows fruit ethylene production and respiration, thus minimizing the effect of a film or coating in terms of modifying the atmosphere inside a fruit.

33.3.1.2.2 Retardation of Weight Loss and Surface Desiccation

Fruits and vegetables also lose water to the surrounding air in the form of water vapor in a process called transpiration. This entails the movement of water from fruit cells to the surrounding atmosphere following a gradient of high water concentration (~100% RH in fruit intercellular spaces or internal atmosphere) to low water concentration (% humidity of the storage environment). For this reason, fresh produce is often stored under conditions of high RH (90–98%) to minimize water loss, subsequent weight loss, and shriveling [78]. Edible coatings can help retard this movement of water vapor, but become more permeable to water vapor and gases under conditions of high RH as explained earlier.

33.3.1.3 Effect on Stability of Lightly Processed Fruits and Vegetables

Light or minimal processing of fresh produce indicates cutting, slicing, coring, peeling, trimming, or sectioning of fruits and vegetables. Since fresh-cut produce, like intact products, have an active metabolism, the processing operations result in a series of chemical and biochemical reactions that can lead to deteriorative changes. These reactions include increased respiration, ethylene production, rapid senescence, undesirable color changes, flavor changes, synthesis of secondary metabolites, and increased microbial growth [79–81]. Many of these reactions are plant wound responses [82–84] due to injury incurred by the minimal processing. Methods used to extend the storage life of lightly processed products are the application of antioxidants, acidulants, preservatives, mineral salts, and osmotic agents. Some applications can be made using an edible coating as a carrier of these active compounds that retard browning or discoloration, microbial growth, and softening, etc. In addition, coatings with the appropriate permeability properties and under certain conditions can create a modified atmosphere around the product and retard respiration, oxidation, and desiccation. Several reviews have been published on this subject [79–81, 85]. Reduction of surface water activity can increase product stability. This can be achieved by infiltration of fruit slices or pieces with fruit juices, sucrose syrups, or glycerol with or without a coating made up of a suitable water-soluble polymeric material [79].

33.3.2 Structural Integrity and Appearance of Coated Products

Coatings on fruits and vegetables can act as lubricants to reduce surface injury, scarring, and chafing [1, 86]. With less wounding of fruit, decay due to opportunist wound pathogens is lessened. In addition, the act of applying certain types of coatings reduces surface microbial populations [87]. For these reasons, waxed citrus fruit experience less decay compared to unwaxed fruit [88]. For food consisting of multiple components, a film can be used to secure the components to the product during marketing [64]. Waxes are also used to encase cheeses to prevent surface molding during the ripening and/or aging process [89]. Resins, zein protein, and microemulsions of waxes can impart a high gloss to the coated product [90, 91]. Shellac, polyethylene, and carnauba wax microemulsions are used on fruits [15], and carnauba, shellac, and zein have been applied to candies and confectioneries as well [16, 38, 92]. Zein has been tested on tomato fruit, but thus far has not been used commercially on fruit [93]. Candelilla wax microemulsions impart a glossy appearance, especially when combined with gelatin protein [94]. Carbohydrate coatings, such as cellulose or pectin, result in an attractive nonsticky sheen when applied to products when dry, but often give an undesirable slippery texture when products become wet with condensation, as is often the case after removal from chilled storage. The polysaccharide film formers, however, do not result in the high gloss finish obtained with shellac, carnauba wax, or zein coatings.

33.4 MATERIALS USED IN EDIBLE COATING AND FILM FORMULATIONS

Many and diverse materials are used in coating or film formulations. Descriptions of the most common main ingredients or film formers are given next. The United States is generally considered the leader in worldwide food regulation, thus, when possible, approval rating based on the U.S. Food and Drug Administration Federal Code of Regulations [95] is also given. Generally recognized as safe (GRAS) status covers direct food additives for their intended use at a quantity not to exceed the amount reasonably required to accomplish the intended physical, nutritional, or other technical effect in food; that are of appropriate food grade; and used with good manufacturing practices (GMP) (FDA, 21 CFR, 1996). The GRAS status is shown without differentiating between initial GRAS status (FDA 21 CFR, Part 182) and reaffirmed as safe with minor restrictions and within specified ranges and uses or purposes and under conditions prescribed (Part 184). Part 180 refers to food additives permitted in food on an interim basis or in contact with food pending additional study. Part

172 contains other direct food additives that are not GRAS including food preservatives, coatings, and films, and special dietary and nutritional additives; Part 173 contains secondary direct food additives including polymer substances and adjuvants for food treatment, enzyme preparations, and specific usage additives; and Part 175 contains indirect food additives including adhesives and components of coatings (FDA 21 CFR [95]).

33.4.1 Lipids

Lipids include a group of hydrophobic compounds, which are neutral esters of glycerol and fatty acids. They also include "waxes," which are esters of long-chain monohydric alcohols and fatty acids [69]. A list of lipid components commonly used in coatings, along with their status according to U.S. FDA 21 CFR [95], is given in Table 33.1. Fatty acids and alcohols lack structural integrity and durability in their free form to be good film formers. Due to the fragile nature of these compounds, lipids are often incorporated into a structural matrix of some other compound such as a polysaccharide [64]. Lipid components are, therefore, incorporated in composite coatings made up of a least two materials. The supporting matrix, if made up of hydrophilic polymers, may affect film resistance to water vapor transmission [96]. Generally, oils are not as resistant to gases and water vapor transfer as are the solid-state waxes [60, 74]. Stearyl alcohol was the most resistant to O_2 transmission, probably due to its ability to crystallize as overlapping platelets with an orientation perpendicular to the direction of gas flow [97, 98]. Generally, coatings that include lipid solids up to 75% can be used to improve coating performance without diminishing moisture-barrier properties, but below 25% solids, permeability increase was observed under test conditions [99].

33.4.1.1 Oils

Paraffin oil, mineral oil, castor oil, rapeseed oil, acetylated monoglycerides, and vegetable oils (peanut, corn, soy) have been used alone or in combination with other ingredients to coat food products. White mineral oil is a petroleum-based product, being a mixture of liquid paraffinic and naphthenic hydrocarbons. It is approved for use as a food-release agent and as a protective coating for fresh fruits and vegetables [69]. Fatty acids and polyglycerides are derived from vegetable or tab oils and are considered GRAS [95]. They are commonly used with glycerides as emulsifiers. Among several oils tested, paraffin oil had the greatest resistance to water followed by vegetable oil and light mineral oil [15]. Acetoglycerides are synthetic fats where acetic acid is substituted for a portion of a naturally occurring fat or oil resulting in mono- or diacetotriglycerides or combinations of these compounds. Although acetic acid is a fatty acid, it does not occur as a glyceride in natural fats. Its water vapor permeability was found to decrease with increasing acetylation [100, 101]. Acetoglycerides are highly flexible in polymorphic form and stable in crystalline form. These modified fats have been used as protective coatings and as plasticizers [102]. Most oils used in coatings are considered direct food additives with varying

TABLE 33.1
Common Lipid Components of Coatings

Lipid	Classification	U.S. FDA 21 CFR #
Oils		
Acetylated monoglyceride	Removable hot-melt strippable coating	172.828, 175.230
Castor oil	Component of coatings for candy, tablets	172.876
Fatty acids from edible sources: capric, lauric, myristic, oleic, palmitic, stearic	Release agent; lubricant; protective coating for raw fruits and vegetables	172.86
Lard oil	GRAS, edible oil	182.7
Mineral oil	Removable hot-melt strippable coating	175.23
Peanut oil	GRAS, edible oil	182.7
Rapeseed oil	GRAS, emulsifier, stabilizer	184.1555
Salts of fatty acids	Binder, emulsifier, anticaking agent	172.863
Soy oil	GRAS, edible oil	182.7
Synthetic isoparaffinic petroleum hydrocarbons	Component of coatings on fruits and vegetables	172.884
Tallow	GRAS, edible oil	182.7
White mineral oil	Component of hot-melt coating for frozen meat, acetylated monoglyceride	172.878
Waxes		
Beeswax	GRAS, surface finishing agent	184.1973
Candelilla	GRAS, lubricant, surface finishing agent	184.1976
Carnauba	GRAS, lubricant, surface finishing agent	184.1978
Paraffin wax	Component of coating	175.250, 175.300
Petroleum wax	Component of microcapsules for flavorings, defoamer, protective coating for cheese, and raw fruits and vegetables	172.886

restrictions according to the U.S. FDA [95]. Paraffin and mineral oil are allowed as release agents and lubricants, defoamers, or components of coating, and castor oil is approved as a release agent and component of coatings. Vegetable oils are generally considered GRAS, while acetylated monoglycerides are considered food additives with few restrictions other than that they be made from edible fat [95].

33.4.1.2 Waxes

Paraffin, carnauba, beeswax, candelilla, and polyethylene waxes have been used to coat food products, alone or in combination with other ingredients. Paraffin wax is a distillate fraction of crude petroleum [103]. Synthetic paraffin wax is formed from the catalytic polymerization of ethylene and is allowed for food use in the United States. It is used as a protective coating for raw fruits, vegetables, and cheese; as a chewing gum base and defoamer; and as a component in the microencapsulation of flavorings [69]. Carnauba wax is the exudate of palm tree leaves from the tree of life (*Copernica cerifera*), found in Brazil. Beeswax or "white wax" is secreted by honeybees, and candelilla wax is an exudate of the candelilla plant (*Euphorbia antisyphilitica*), which is a reedlike plant that grows in Mexico and southern Texas. These natural waxes are considered GRAS [95] in the United States and are used in chewing gum, hard candy, and edible coatings. Polyethylene wax is oxidized polyethylene or the basic resin produced by the mild oxidation of polyethylene, a petroleum-based product. This substance is allowed for use in edible coatings for fruits and nuts where the peel or shell is not normally ingested [69, 95, 104]. Coatings made with this wax are more permeable to gases than shellac and most other polymers [104].

33.4.1.3 Emulsions

Emulsion coatings are oil or wax dispersed in water or some other hydrophilic solution. A macroemulsion has dispersed wax particle sizes ranging from 2×10^3 to 1×10^5 Å, and microemulsions from 1×10^3 to 2×10^3 Å. Melted wax disperses in water in a manner similar to oil [105]. Carnauba wax and beeswax form stable microemulsions with the appropriate emulsifiers, forming a glossy coating, while macroemulsions generally impart little shine to the coated product [69].

33.4.2 Resins

Resins are a group of acidic substances that are produced and secreted as a wound response by specialized plant cells of trees and shrubs. Synthetic resins are petroleum-based products [69]. A list of resins commonly used in coatings, along with their status according to U.S. FDA 21 CFR [95], is shown in Table 33.2.

33.4.2.1 Shellac

Shellac resin is secreted by the insect *Laccifer lacca* found in India. Shellac is composed of aleuritic and shelloic acids [106], is compatible with waxes, and gives coated products a high gloss appearance. This compound is permitted as an indirect food additive (FDA 21 CFR 175.300: resinous and polymeric coatings for food contact surfaces), but is nevertheless commonly used in coatings for fresh fruits and candies where the coated surface is consumed. Apparently, this is allowed because a petition has been submitted for GRAS status [69]. Shellac and other resins have relatively low permeability to gases and moderate permeability to water vapor [104, 107].

TABLE 33.2
Common Resin Components of Coatings

Resins	Classification	U.S. FDA 21 CFR #
Copal	Resinous and polymeric coatings	175.300
Coumarone indene	Resinous and polymeric coatings	172.215
Damar	Resinous and polymeric coatings	175.300
Elemi	Resinous and polymeric coatings	175.300
Shellac	Resinous and polymeric coatings	175.300
Terpene	Moisture barrier on soft gelatin capsules or powders of ascorbic acid	172.280
Wood rosin	Coatings on fresh citrus fruit	175.300

33.4.2.2 Wood Rosin

Wood rosin is manufactured from oleoresins of pine trees. The rosin is the residue left after distillation of volatiles from the crude resin [69]. It is less expensive than shellac, and specific esters of maleic-anhydride-modified wood rosin are approved for use in coatings for citrus. Similarly, coumarone–indene resin is approved for use specifically on citrus fruit. Coumarone–indene resin is a petroleum and/or coal tar by-product that is available in several grades. It is most often used as a component of the "solvent waxes" for citrus [69, 108]. These so-called waxes consist mostly of resins with little or no actual wax component and small amounts of petroleum solvent (U.S. FDA 21 CFR 172.250), ethanol (U.S. FDA 21 CFR 184.1293), or isopropanol (U.S. FDA 21 CFR 173.240, 173.340) [3, 69, 95]. They have low viscosity and rapid drying rates. Resin solution waxes contain a small amount of lipid (usually oleic acid), morpholine (U.S. FDA 21 CFR 172.235) or potassium or ammonium hydroxide (U.S. FDA 21 CFR 184.1631 and 184.1139, respectively), and other ingredients [95, 109, 110].

33.4.2.3 Other Resins

Terpene resin is obtained from polymerization of terpene hydrocarbons derived from wood and is approved as a direct food additive [95]. It is allowed for use as a moisture barrier in soft gelatin capsules. Other resins allowed only for food

contact include copal, damar, and elemi, which are used in the pharmaceutical industry [69, 95].

33.4.3 Proteins

Proteins have been used for their film-forming abilities for nonfood applications since ancient times as a component of glue, paint, leather finishes, paper coatings, and inks. More recently protein materials, such as the milk protein casein and corn protein zein, have been used as edible coatings for extruded meats as well as nuts and confectionery items, respectively [111]. A list of proteins commonly used in coatings and films, along with their status according to the U.S. FDA 21 CFR [95], is given in Table 33.3. Film-forming proteins of plant origin include corn zein, wheat gluten, soy protein, peanut protein, and cottonseed protein, of which all but the latter are considered GRAS [95]. Keratin, collagen, gelatin, casein, and milk whey proteins are film formers derived from animal sources, of which casein and whey proteins are GRAS [95]. Adjustment of protein film pH can alter film formation and permeability properties, as was demonstrated for soy protein, wheat gluten [112, 113], and casein [70]. Most protein films are hydrophilic and, therefore, do not present good barriers to moisture. However, dry protein films such as zein, wheat gluten, and soy present relatively low permeabilities to O_2 [112].

TABLE 33.3
Commonly Used Protein Materials in Coatings

Protein Materials	Classification	U.S. FDA 21 CFR #
Casein/Sodium caseinate	GRAS, GMP	182.90, 182.1748
Collagen		
Cottonseed (modified products)	Food additive	172.894
Gelatin	Microcapsules for flavorings (succinylated gelatin)	172.230
Fish protein concentrate	Food supplement	172.385
Fish protein isolate keratin	Food supplement	172.340
Peptones	GRAS, nutrient supplements	184.1553
Soy protein isolate	Migrating to food from paper products	182.90
Wheat gluten	GRAS, stabilizer, thickener, surface finishing agent	184.1322
Whey	GRAS, GMP	184.1979
Zein	GRAS, surface finishing agent	184.1984

Note: GRAS, generally recognized as safe by the U.S. Food and Drug Administration; GMP, good manufacturing practices.

33.4.3.1 Milk Proteins

Milk protein products include casein (80% of total milk protein) and whey (20% of total milk protein) and combinations of both [65, 114]. They can result in films of different properties depending on the commercial source and method of extraction [70, 114].

33.4.3.1.1 Casein

Caseins are soluble in aqueous solutions and form flexible colorless films. The addition of lipid compounds and adjustment of pH reduced the water vapor permeability of casein films [115], while the addition of whole milk, sodium caseinate, and nonfat dry milk or whey into polysaccharide films decreased the water vapor permeability of these hydrophilic films [116]. Lactic-acid-treated casein films retained more sorbic acid preservative, improving the microbial stability of dehydrated apricot and papaya in intermediate-moisture food test systems [117].

33.4.3.1.2 Whey

Whey proteins produce films similar to those produced from caseinates. Heating is required to form intermolecular disulfide bonds, which produces films that are water-insoluble and brittle, requiring plasticizers [111, 114].

33.4.3.2 Collagen and Gelatin

Collagen is the major component of skin, tendon, and connective tissues in animals [111]. This material is partially digested with acid or enzymes to produce edible collagen casings. Collagen casings for meat products were one of the first examples of edible film application in modern times. Gelatin is formed from the partial hydrolysis of collagen [111] and is also allowed as a component of microcapsules for flavorings and for soft capsules in the pharmaceutical industry [95]. It is soluble in aqueous solutions, forming a flexible, clear, oxygen-permeable film.

33.4.3.3 Wheat Gluten

The gluten complex is a combination of gliadin and glutenin polypeptides with some lipid and carbohydrate components [111, 118]. It is soluble in aqueous alcohol, but alkaline or acidic conditions are required for the formation of homogeneous film-forming solutions [112]. This material also requires plasticizers to increase flexibility, for the films are excessively brittle [111, 118]. These films have high water permeability but are good barriers to O_2 and CO_2 [118].

33.4.3.4 Corn Zein

Zein is a prolamine derived from corn gluten and is insoluble in water except at very low or high pH. This is due to its high content of nonpolar amino acids. It is soluble in aqueous alcohol and dries to a glossy grease-resistant surface. The film is, however, brittle, and plasticizers are required to add plasticity [111, 118]. It has been used as a substitute for shellac because of its high-gloss appearance, faster drying rate, and increased stability during storage [118].

33.4.3.5 Soy Protein

Soy protein is available as a concentrate (70% protein) or an isolate (90% protein). Film formation is enhanced by heating, which partially denatures the protein, allowing the formation of disulfide bonds. This was shown to lower water vapor permeability. Enzymatic digestion can increase cross-linking [119]. The pH must be adjusted away from the isoelectric point of the soy protein (~4.6) in order to form films. In Asia, films are formed from heated soy milk and are used for wrapping food products [111].

33.4.3.6 Peanut Protein

Peanut protein films can be formed by two methods. The first is surface film formation, using protein/lipid solutions derived from roasted peanut, partially defatted peanut flour, and protein concentrate. This produces films with rough surface texture and poor mechanical properties. Films can also be produced by the deposition method at pH 9.5 from peanut protein concentrate. This method showed promise for development as an edible film [120].

Zein, casein, and soy proteins have been used on confectionery, fruit, and vegetable products as well as on eggs. Self-supporting sheets of edible proteins have been developed that dissolve in water for microencapsulation of flavorings. Casein, soy [121], peanut, corn protein, and collagen have been used to form free-standing films or sheets for wrapping of foodstuffs [111]. Films made of proteins can add a nutritional component to coated foods especially if formulated to include diet-limiting amino acids [111, 118]. Allergies to food proteins can be a concern when developing coatings and films from these materials. Gluten intolerance and allergic reactions to milk proteins (casein and whey) and associated lactose are common and may require labeling.

33.4.4 Carbohydrates

Polysaccharides are used in food systems as thickeners, stabilizers, gelling agents, and emulsifiers [122]. They comprise an abundant and renewable resource of hydrophilic film-forming agents with a wide range of viscosities, relatively low permeability to gases, but little resistance to water vapor transfer. A list of commonly used polysaccharides in coatings and films, along with their status according to the U.S. FDA 21 CFR [95], is given in Table 33.4.

33.4.4.1 Cellulose

Cellulose is the most abundant polysaccharide on the planet, being a major component of plant cell walls. Cellulose is made up of repeating glucose units in β-1,4-linkage. In its natural form, cellulose is not soluble in water, but derivatized forms such as sodium carboxymethyl cellulose (CMC), methylcellulose (MC), hydroxypropyl cellulose (HPC), and hydroxypropyl methylcellulose (HPMC) are more soluble [102, 122]. These derivatives have different permeabilities to water vapor and gases and are good film formers [102, 105]. The degree of substitution and type of substitution (ionic and nonionic)

TABLE 33.4
Commonly Used Polysaccharides in Coatings

Polysaccharides	Classification	U.S. FDA 21 CFR #
Agar	GRAS, drying and flavoring agent, stabilizer, thickeners, surface finishing	184.1115
Alginate	GRAS, emulsifier, stabilizer, thickener	184.1011
Carrageenan	Emulsifier, stabilizer, thickener, gelling agent	172.620
Salts of carrageenan	Emulsifier, stabilizer, thickener	172.626
Chitosan	Approved in Canada	
Dextrin	GRAS, formulation aid, processing aid, stabilizer, thickener	184.1277
Ethyl cellulose	Binder, filler, component of protective coatings for vitamin and mineral tablets	172.868
Furcellaran	Emulsifier, stabilizer, thickener	172.655
Salts of furcellaran	Emulsifier, stabilizer, thickener	172.660
Gellan gum	Stabilizer, thickener	172.665
Gum Arabic (acacia gum)	GRAS, emulsifier, formulation aid	184.1330
Gum ghatti	GRAS, emulsifier	184.1333
Gum karaya	GRAS, formulation aid, stabilizer, thickener	184.1349
Gum tragacanth	GRAS, emulsifier, formulation aid	184.1351
Locust bean gum	GRAS, stabilizer, thickener	184.1343
Guar gum	GRAS, emulsifier, formulation air, firming agent	184.1339
Hydroxypropyl cellulose	Emulsifier, film former, protective colloid, thickener	172.870
Hydroxypropyl methylcellulose	Emulsifier, film former, protective colloid, thickener	172.874
Methylcellulose	GRAS, GMP	182.1480
Methyl ethyl cellulose	Aerating, emulsifying or foaming agent	172.872
Modified starch	Food additive	172.892
Pectins	GRAS, GMP	184.1588
Sodium carboxymethyl cellulose	GRAS, GMP	182.1745
Xanthan gum	Stabilizer, emulsifier, thickener, suspending agent	172.695

Note: GRAS, generally recognized as safe by the U.S. Food and Drug Administration; GMP, good manufacturing practices.

for functional groups and polymer chain length affect permeability, solubility, and viscometric properties [122, 123]. The derivatives CMC and MC are GRAS, while HPC and HPMC are approved as direct food additives for the purpose of film former, stabilizer, thickener, and suspending agent [95]. The latter two derivatives are not permitted for food use in all countries. However, several commercial coatings were developed from cellulose polymers including TAL Pro-long (Coutaulds Group, London), Semperfresh (United Agri Products, Greeley, Colorado), and Nature Seal (EcoScience Corp., Orlando, Florida). Another cellulose product is called cellulon fiber, which is bacterial cellulose produced by aerobic fermentation of glucose by a strain of *Acetobacter*. It has a fine fiber structure physically but is not chemically different from plant cellulose. It has been applied to surimi to aid in the binding of water [124] but has no reported uses as a coating or surface agent.

33.4.4.2 Pectin

Pectins are a complex mixture of polysaccharides that are also components of plant cell walls [125]. They are commercially obtained from citrus peel and apple pomace [123]. These polymers are mainly long chains of α-1,4-linked galacturonic acid units with varying degrees of esterification with methyl groups. The degree of esterification (DE) affects solubility and gelation properties; pectins with DE above 50% are labeled high-methoxyl and below 50% DE, low-methoxyl pectins [102, 123]. As with cellulose polymers, the chain length also affects solubility and viscosity. When used as a film former in coatings, this polymer gives a somewhat glossy, nonsticky surface, and LM pectins can be cross-linked with calcium ions to form gels [102]. Coatings made with pectin materials generally have high water vapor transmission rates [126] due to their hydrophilic nature [127], which can be improved by the addition of paraffin or beeswax. The tensile strength of pectinic acid films increases with a decrease in methoxyl content [128] because the removal of ester groups leads to increased cross-bonding between residual carboxyl groups. Miers et al. [128] reported that pectin coatings were of acceptable strength with methoxyl contents of 4% or less and intrinsic viscosities of 3.5 or above. Pectins are generally considered GRAS [95].

33.4.4.3 Chitin/Chitosan

Next to cellulose, chitin is the second-most abundant polysaccharide on the planet, being a component of fungal and green algae cell walls and the skeletal substance of invertebrates [129]. It is a β-1,4-linked polymer of 2-acetamido-2-deoxy-D-glucan [125]. Partial deacetylation of chitin results in chitosan, which has been shown to induce plant-defense responses and inhibit the growth of fungi [130, 131]. Use of this polymer as a film former and natural preservative resulted in the commercial coating Nutri-Save (Nova Chem, Halifax, Nova Scotia, Canada). Methylation of the polymer resulted in a twofold resistance to CO_2 [15, 132]. This allows it to retard the ripening of climacteric fruits [133]. It, however, has relatively low resistance to water vapor transfer compared to lipid materials. Chitosan has not yet received approval for food use in the United States (although approved for supplements), but it is approved in Canada. The antimicrobial activity of chitosan was found to increase with ionic strength but decrease with addition of metal ions. The antibacterial activity of chitosan was dependent on its charges and solubility [134].

33.4.4.4 Starch

Amylaceous materials (amylose, amylopectin, and derivatives) have also been used to make coatings. These films have been reported to be semipermeable to CO_2 but highly resistant to O_2 [135]. Most starch consists of 25% amylose and 75% amylopectin, with one notable exception being hybrid corn, which contains 50–80% amylose. Amylose is a polymer of α-1,4-linked glucose, and amylopectin consists of an amylose backbone with side chains of α-1,6-linked glucose. Of the two polymers, amylose is a better film former and amylopectin is more useful as a thickening agent. Some derivatives, such as a hydroxypropyl amylose, showed low permeability to O_2, improved water solubility [102], and increased elongation properties, but no resistance to water vapor. Dextrins (partially hydrolyzed starch molecules, i.e., reduced in size as measured by dextrin equivalent or DE) are used as film formers, encapsulating agents, and flavor carriers. Coatings made from such polymers have lower permeability to water vapor compared to starch films [102, 136] and may have resistance to O_2 [137]. Carboxylated dextrins are used as encapsulating agents [125]. Raw starch and dextrin products are considered GRAS, while modified starch products (modified by acid, bleach, esterification, or oxidized using chlorine) are approved as direct food additives [95].

33.4.4.5 Aloe Vera

Aloe vera gel was used to coat table grapes and extended shelf life by 35 days at 1°C. The gel worked as a barrier to O_2 and CO_2, creating a modified atmosphere, and acted as a moisture barrier and thus reduced weight loss, browning, softening, and growth of yeast and molds. The material reportedly contains antimicrobial compounds and thus prevents decay. This material has been used as a functional ingredient in beverages for years [138]. Aloe vera contains malic-acid-acetylated carbohydrates (including β-1,4-glucomannans) that demonstrated anti-inflammatory activity [139].

33.4.4.6 Konjac Glucomannan

Konjac glucomannan is a polysaccharide derived from the konjac tuber and consists of 1,4-linked β-mannose and β-glucose units with some acetyl groups. It has been used in food, film formers, and biomedical applications. It was combined with chitosan and nisin (used by the food industry as a preservative) and was found to be effective against pathogenic bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* [140].

33.4.4.7 Seaweed and Gum Polymers

33.4.4.7.1 Seaweed Products

Seaweed products such as carrageenan, alginates, and agar make good film formers or gels. Carrageenan consists of sulfate esters of 3,6-anhydro-α-D-galactopyranosyl units

[123, 141], alginates are salts of alginic acid (1,4-polyuronic acid with poly-β-D-mannopyranosylurinic and poly-α-L-gulopyranosyluronic acid blocks) [102, 123, 125], and agar is made up of β-1,4-D-galactopyranosyl linked to 3,6-anhydro-α-L-galactopyranosyl, partially esterified with sulfuric acid. Of these three, agar is more for formation of gels (currently used as a culture medium) and the other two polymers as gel and film formers. Alginate gels are relatively heat-stable [123]. Carrageenan is approved as a direct food additive as an emulsifier, stabilizer, and thickener, while agar is GRAS [95]. In Japan, there is a commercial carrageenan-based coating called Soageena (Mitsubishi International Corp., Tokyo).

33.4.4.7.2 Gum Products

Certain gum products are exudates from plants found mostly in Africa and Asia produced in response to injury [123, 136, 143], some of which are seed and fermentation products. Gums are complex heteropolysaccharides including gum arabic or acacia gum from the tree *Acacia senegal* and related species (*n*-galactopyranosyl, L-rhamnopyranosyl, L-arabinopyranosyl, L-arabinofuranosyl, and D-glucopyranosyluronic acid units with calcium, magnesium, and potassium ions) [144, 145]. Gum arabic forms an aqueous solution of low viscosity and can form stable emulsions with most oils [125]. It is used in the confectionery industry as a stabilizer, adhesive, and flavor fixative. It has also been used to coat pecans [146] and is a good emulsifier [123].

Other less-used gums include gum tragacanth, whose film-forming properties are more useful in nonfood products such as hair and hand lotions and creams [125]. Gum karaya, which forms smooth films that require plasticizers; locust bean gum, whose film-forming properties have been used in the textile industry as a finishing agent and as a common component of cosmetics, sauces, and salad dressings; guar gum, which is also used as a film former in the textile industry (both locust bean and guar gum are galactomannans) [123]; xanthan gum, a fermentation product with a cellulosic backbone [123], used as a thickener in sauces, gravies, frostings, fruit gels, and coatings to prevent moisture migration during frying [125] and in salad dressings with propylene glycol alginate [147]; and gellan gum, another fermentation product developed and patented by Kelco [148], which is also used in glazes, icings, and jams/jellies [125]. Of these gums, gum arabic (acacia gum), gum ghatti, guar gum, and locust bean gum (carob gum) are considered GRAS, while xanthan and gellan gum are approved as direct food additives for use in glazes, icings, frostings, jams, and jellies, and as a stabilizer, emulsifier, thickener, etc. [95].

33.5 AS INDIVIDUAL TREATMENTS OR IN COATING FORMULATIONS

Materials other than film formers are added to edible coatings for two main reasons. One is to improve the structural, mechanical, or handling properties of a coating. The other is to improve the quality, flavor, color, or nutritional properties of the coated product [62]. In the latter case, the coating acts as a carrier of useful compounds that have a desired effect on the coated item.

33.5.1 Plasticizers, Emulsifiers, and Surfactants

33.5.1.1 Plasticizers

Plasticizers are usually low molecular weight compounds that impart increased strength and flexibility to coatings, but also increase coating permeability to water vapor and gases [64, 102]. Commonly used plasticizers, along with their status according to the U.S. FDA 21 CFR [95], are listed in Table 33.5. Common plasticizers include polyols such as glycerol, sorbitol, mannitol, propylene glycol, and polyethylene glycol (molecular weight 200–9500). Sucrose, sucrose fatty acid esters, and acetylated monoglycerides also can be used as plasticizers. Of these, glycerol, sorbitol, and propylene glycol are considered GRAS [95].

33.5.1.2 Emulsifiers and Surfactants

Emulsifiers can be classified as surface-active agents or as macromolecular stabilizers. Macromolecular stabilizers are proteins, gums, and starches that stabilize emulsions [149]. Commonly used emulsifiers and surfactants, along with their status according to the U.S. FDA 21 CFR [95], are listed in Table 33.5. Surface-active agents reduce surface water activity and can affect the rate of moisture loss from a food when used as a coating. This was shown with glycerol monopalmitate and glycerol monostearate and other 16- to 18-carbon fatty alcohols [102]. Reduction of surface water activity at the water/oil interface helps to both form and stabilize emulsions, which is important for shelf-life properties of emulsion coatings. The hydrophilic–lipophilic balance (HLB) of surfactants ranks these compounds according to their hydrophobic and hydrophilic portions, which has an effect on their performance as emulsifiers. For example, sodium lauryl sulfate is a very hydrophilic surfactant with a HLB value of 40. Generally, surfactants with low HLB values are effective for water-in-oil emulsions, and those with high HLB values are more useful for oil-in-water emulsions [69]. Some common emulsifiers are acetylated monoglyceride, lecithin (GRAS) and lecithin derivatives, ethylene glycol monostearate, glycerol monostearate, sorbitan fatty acid esters (TWEENS), and palm and corn oil (GRAS) [95]. Surfactants help coatings adhere to coated surfaces. Most natural waxes also have emulsifying properties since they are comprised of long-chain alcohols and esters [69].

33.5.2 Fungicides and Biocontrol Agents

33.5.2.1 Fungicides

Fresh fruits and vegetables are susceptible to a variety of postharvest decay types that can be reduced by treatment with fungicides with and without a coating or "wax" [150]. About 20 compounds have been developed and tested for use as postharvest pesticides over the last 30 years [62], but

TABLE 33.5
Commonly Used Plasticizers, Emulsifiers, and Surfactants

Compounds	Classification	U.S. FDA 21 CFR #
Acetylated monoglycerides	Emulsifiers, component of coating	172.828
Corn oil	Edible oil	
Ethoxylated mono-, diglycerides	Emulsifiers	172.834
Glycerol	GRAS, GMP	182.1320
Glycerol monopalmitate (ethoxylated mono- and diglycerides)	Emulsifier	172.834
Glycerol monostearate (ethoxylated mono- and diglycerides)	Emulsifier	172.834
Hydroxylated lecithin	Emulsifier	172.814
Lecithin	GRAS, GMP	184.1400
Mannitol	Permitted food additive, component of resinous and polymeric coatings	180.25, 175.300
Oleic acid	Lubricant, binder, defoaming agent	172.862
Palm oil	GRAS, cocoa butter substitute	184.1259
Polyethylene glycol	MW 2200–9500; coating, binder, plasticizer, lubricant	172.820
Polysorbate 60	Emulsifier, foaming agent	172.836
Polysorbate 65	Emulsifier	172.838
Polysorbate 80	Emulsifier, dispersing agent, surfactant, wetting agent	172.840
Propylene glycol	GRAS, solvent, thickener, component of resinous and polymeric coatings	184.1666, 175.300
Propylene glycol alginate	Component of coatings for citrus	172.212
Sodium lauryl sulfate	Emulsifier, whipping agent, surfactant, wetting agent	172.822
Sodium stearoyl lactylate	Surfactant, emulsifier, stabilizer	172.846
Sorbitan monooleate	Emulsifier for clarification of cane or beet sugar juice	173.110
Sorbitan monostearate	Emulsifier	172.842
Sorbitol	Component of resinous and polymeric coatings	175.300
Sucrose	GRAS, GMP	184.1854
Sucrose fatty acid esters	Emulsifiers, texturizers, components of protective coatings for fresh fruit	172.859

Note: GRAS, Generally recognized as safe by the U.S. Food and Drug Administration; GMP, good manufacturing practices.

many have been banned or not reregistered in the United States and other nations. Use of fungicides applied in fruit coatings has been reported for citrus including benomyl, imazalil, and thiabendazole (TBZ), of which only the latter two are currently registered for use on citrus in the United States. These fungicides are applied in solvent or water waxes, and this results in reduced ability to inhibit mold growth compared to application as an aqueous suspension. It is thought that encapsulation of the fungicide in the wax is the reason for its reduced efficiency [53]. Use of fungicides in fruit coatings has been reported for stone fruits (methyl-1-(butylcarb amoyl)-2-benzimidazolecarbamate or benomyl) [49], papayas (TBZ) [54], strawberries (3-(3,5-dichlorophenyl)-*N*-(1-methyl ethyl)-2,4-dioxo-1-imidazolidinecarboxamide, iprodione or Rovral® [55], tomatoes (*N*-[(trichloromethyl)thio]-4-cyclohex ene-1,2-dicarboximide or captan) [151], and apples (Rovral) [152], as well as captan and benomyl on raspberries [153].

33.5.2.2 Biological Control Agents

Antagonistic yeasts and bacteria have been shown to inhibit mold growth and thus prolong the shelf life of fresh fruits and vegetables [154, 155]. The mechanisms of action are reported to be the production of an antibiotic compound, competition for nutrients at wound sites on fresh produce, direct interaction with the pathogen, and induction of host defense responses [155]. These compounds have been successfully applied in fruit coatings and were shown to delay spoilage of citrus fruit by this method [156, 157]. Two commercial products approved and available on the U.S. market are Biosave® (EcoScience Corp., Orlando, Florida), which contains an antagonist bacteria (*Pseudomonas syringae*), and Aspire® (Ecogen Corp., Langhorne, Pennsylvania), which contains an antagonist yeast (*Candida oleophila*) for control of decay on apples and citrus fruits.

33.5.3 Preservatives

Chemical preservatives such as salt, nitrites, and sulfites have long been used to prolong the shelf life of food products [36]. Coatings can also act as carriers of antimicrobial agents for lightly processed and processed food products [62]. Commonly used preservatives, along with their U.S. FDA 21 CFR [95], are listed in Table 33.6.

33.5.3.1 Benzoates, Sorbates, and Other Short-Chain Organic Acids

Preservatives such as benzoic acid and benzoates are most effective at pH 2.5–4.0 with the undissociated form of benzoic acid being most effective (pK_a 4.2), rendering this preservative ineffective above pH 4.5 [36]. This preservative controls yeasts and molds more effectively than bacteria and is considered GRAS to a maximum of 0.1% in the United States and up to 0.15–0.25% in other countries [95]. Sorbic acid and sorbates are also most effective in the undissociated state against fungi and certain bacteria [36]. This preservative is also considered GRAS for most products in accordance

TABLE 33.6
Commonly Used Preservatives

Preservatives	Classification	U.S. FDA 21 CFR #
Acetic acid	GRAS, curing or pickling agent, or in food at levels not to exceed GMP	184.1005
Benzoic acid	GRAS, antimicrobial agent	184.1021
Calcium disodium EDTA	Preservative, color retention	172.120
Citric acid	GRAS, GMP	184.103
Dehydroacetic acid	Preservative for cut or peeled squash	172.130
Fumaric acid	Nutritional additive	172.350
Lactic acid	GRAS, antimicrobial agent	184.1061
Methylparaben	GRAS, antimicrobial agent	184.1490
Natamycin	Mold inhibitor for sliced cheeses	172.155
Potassium sorbate	GRAS, GMP	182.3640
Propionic acid	GRAS, antimicrobial agent	184.1061
Propylparaben	GRAS, antimicrobial agent	184.1670
Sodium benzoate	GRAS, antimicrobial agent	184.1733
Sodium nitrate	Preservative, color fixative for fish and meat	172.170
Sodium nitrite	Preservative, color fixative for fish and meat	172.175
Sorbic acid	GRAS, GMP	182.3089

Note: GRAS, Generally recognized as safe by the U.S. Food and Drug Administration; GMP, good manufacturing practices.

with good manufacturing practices up to a level of 0.1% in the United States [95]. Sorbates are permitted in all countries of the world for preservation of various food products in the range of 0.15–0.25%. Acetic, lactic, propionic, fumaric, and citric acids also can be used in coatings and contribute to antimicrobial activity. Use of coatings as carriers of preservatives such as benzoates and sorbates improved their performance when applied to cut fruit or cheese analogs. This may be due to the prevention of diffusion of preservatives into the food tissue or the fact that more preservative is present on the cut surface due to the thickness of the coating. Use of coatings to establish a surface pH that favors the active form of sorbic acid and other preservatives is also a possibility. This was demonstrated with a zein coating on a cheese analog for sorbate [158]; with MC/fatty acid films with sorbate in a test system [46, 159]; with chitosan, MC, and HPMC films with sorbate in a test system; and with a CMC/soy protein coating on cut apple with sorbate and benzoate [41].

33.5.3.2 Parabens

Alkyl esters of p-hydroxybenzoic acid, or parabens, are effective antimicrobial agents, especially against yeasts and molds. In the United States, methyl and propyl parabens are considered GRAS up to 0.1% [95]. In the United Kingdom, methyl, ethyl, and propyl parabens are permitted in food, while other countries allow butyl ester parabens as well [36].

33.5.3.3 Sulfites

Sulfites or sulfur dioxide and its various salts are effective antimicrobials for the control of yeasts, molds, and especially bacteria, and they prevent enzymatic browning in foods. The effectiveness of this preservative is greatest when the acid is undissociated at pH<4 (pK_a of sulfur dioxide = 1.76 and 7.20). Although sulfur dioxide and various sulfite salts are considered GRAS in the United States, they cannot be used on meats, in food products that are sources of thiamine, or on raw fruits and vegetables [95] due to the elicitation of allergenic responses in a certain segment of the population [36].

33.5.3.4 Sucrose Esters and Chitosan

Sucrose esters are approved as emulsifiers and are an ingredient in edible coatings along with cellulose in Tal Pro-long (Courtaulds Group) and Semperfresh (United Agri Products), or with guar gum in Nu-coat Flo (Surface Systems International Ltd.). Sucrose esterified with palmitic and stearic acids showed some antimicrobial activity against certain molds at levels of 1%. Chitosan, the film former of the edible coating Nutri-Save (Nova Chem. Ltd.), has been shown to inhibit growth of fungi on plants by inducing plant defense responses [130, 131].

33.5.3.5 Other Natural Antifungal Compounds

Unripe fruits are often more resistant to decay possibly due to the presence of some antifungal compounds. Several of these compounds were found in unripe mango including 5,12-cis-heptadecenyl resorcinol and 5-pentadecenyl resorcinol. A similar situation was discovered in unripe avocado with the antifungal compounds 1-acetoxy-2-hydroxy-4-oxohenei cosa-12,15-diene and 1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene. High levels of CO_2-enhanced concentrations of the antifungal avocado diene in treated fruits [160] present a possible explanation for the antimicrobial action of these compounds, at least in the case of avocado fruit.

An antifungal essential oil and a long-chain alcohol, thought to be 17-pentatriacontanol, showed antifungal activity by inhibiting the mycelial growth of *Aspergillus carneus.* This compound was isolated from *Achyranthes aspera,* an herb with reported medicinal properties [161]. Isothiocyanates derived from mustard and horseradish have been shown to have antimicrobial activity [162]. Another compound, pyrrolnitrin, was isolated from the bacteria *Pseudomonas cepacia,* which in turn was isolated from apple leaves. This microbe was found to have antagonistic activity toward *Penicillium expansum*, *Botrytis cinerea*, and *Mucor* species due to the production of the secondary metabolite pyrrolnitrin. This compound was applied to harvested strawberries and was found to retard various storage rots [163]. None of these natural fungicides have been approved for human consumption, but these offer promising alternatives to synthetic fungicides and preservatives.

33.5.4 Antioxidants

Antioxidants are compounds that inhibit or prevent the oxidation reaction caused by free radicals, with or without oxidation enzymes, that cause discoloration or browning of certain

TABLE 33.7
Commonly Used Antioxidants

Antioxidants	Classification	U.S. FDA 21 CFR #
Anoxomer	Antioxidant	172.105
Ascorbic acid	GRAS, GMP	182.8013, 182.5013
Ascorbic acid-2-phosphate		
Ascorbic acid-3-phosphate		
Ascorbyl palmitate	GRAS, GMP	182.3149, 172.110
BHA	GRAS, GMP	182.3169, 172.115
BHT	GRAS, GMP	182.3173
L-Cysteine	GRAS, improved biological quality of total protein in a food	184.1271, 172.320
Diphenylamine (DPA)	Surface treatment of apples for scald disorder	
Erythorbic acid	GRAS, GMP	182.3041
Ethoxyquin	Antioxidant	172.140
4-Hydrocymethyl-2-6-di-tert-butylphenol	Antioxidant	172.150
Lecithin	GRAS, GMP	184.140
Potassium bisulfite	GRAS, GMP, raw fruits and vegetables	182.3616
Potassium metabisulfite	GRAS, GMP, raw fruits and vegetables	182.3637
Propyl gallate	GRAS, antioxidant	184.1660
Rosemary	GRAS, flavoring	182.10, 182.20
Sodium bisulfite	GRAS, GMP, raw fruits and vegetables	182.3739
Sodium metabisulfite	GRAS, GMP, raw fruits and vegetables	182.3766
Sodium sulfite	GRAS, GMP, raw fruits and vegetables	182.3798
TBHP	Antioxidant	172.190
TBHQ	Antioxidant	172.185
Tocopherols	GRAS, GMP	182.8890
α-Tocopherols	GRAS, inhibitors of nitrosamine formation	184.1890
α-Tocopherol acetate	GRAS, GMP	182.8892

Note: GRAS, Generally recognized as safe by the U.S. Food and Drug Administration; GMP, good manufacturing practices.

fruit and vegetable tissues and rancidity of fats [17, 18]. This can affect the color or flavor of meat, fish, mushrooms, fruit, and vegetable products. Commonly used antioxidants, along with their U.S. FDA 21 CFR [95], are listed in Table 33.7.

33.5.4.1 Phenolic Antioxidants

The phenolic structure of certain compounds suppresses free radical formation, which delays the autooxidative process in fat or oil by acting as a proton donor [18]. Approved phenolic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and esters of gallic acid such as propyl gallate and tertiary butyl hydroquinone (TBHQ). Natural antioxidants are also effective, such as the tocopherols and lecithin. The antioxidants BHA, BHT, tocopherol, and lecithin are GRAS, while TBHQ is approved as a direct food additive and propyl gallate as an indirect food additive component of coatings [95]. These antioxidants are also approved for food use in many countries, especially BHA, BHT, and tocopherol [18]. Coatings have been used as carriers of antioxidants to retard rancidity of meat and nut products, and discoloration of lightly processed fruits and vegetables [16, 39–41]. For whole apples, aqueous dips in the antioxidant diphenylamine (DPA) (300–3000 ppm) or ethoxyquin help to reduce surface discoloration known as scald [152, 164].

33.5.4.2 Other Antioxidants and Antibrowning Agents

Some agents such as cinnamic and benzoic acids (both GRAS) [95] are effective browning inhibitors in combination with ascorbic acid since, like sulfites, they inhibit polyphenol oxidase (PPO) activity [21]. This enzyme is responsible for the browning that occurs when monophenolic compounds of plants or shellfish are hydroxylated to o-diphenols and subsequently to o-quinones in the presence of oxygen and PPO in plants and shellfish. The PPO enzyme requires copper, and thus complexing and chelating agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid can inhibit enzymatic browning [17]. Ascorbic acid and its derivatives, erythorbic acid, and ascorbic acid-2-phosphate and -triphosphate, are effective inhibitors of enzymatic browning for cut apple [21, 165].

Ascorbyl palmitate, cinnamic acid, benzoic acid, and ~3-cyclodextrin were reported to be effective browning inhibitors in juice [21]. Ascorbic acid, erythorbic acid, and ascorbyl palmitate are GRAS [95], but other ascorbic acid derivatives are, so far, not approved. The amino acid cysteine is also an effective inhibitor of PPO [166]. Rosemary extract (and its constituents carnosol, carnosic acid, and rosmarinic acid) is a source of natural antioxidants [167, 168]. Citric acid and EDTA have been incorporated into coatings as browning inhibitors for cut apples, potatoes, and mushrooms [41, 169]. The amino acid cysteine inhibits PPO activity by reacting with quinone intermediates as well as reduced glutathione. Inorganic halides such as sodium or calcium chloride ($CaC1_2$) also inhibit PPO activity [17]. Resorcinol and its derivatives, such as 4-hexyiresorcinol, inhibit browning tyrosinase isozymes in mushrooms and may inhibit PPO by serving as a substrate for this enzyme. These compounds have antimicrobial activity as well [22]. They are not yet approved for food use in the United States.

33.5.5 Mineral and Growth Regulator Treatments

33.5.5.1 Calcium

The mineral calcium has many postharvest uses [31]. Postharvest dips of calcium chloride ($CaC1_2$) can reduce symptoms of bitter pit or small brown lesions that occur on apples as well as scald [152, 170]. Calcium or $CaC1_2$ dips or infiltrations on whole or cut fruit has been reported to increase fruit firmness for apple [32, 171], peach, [172], blueberry [33],

and strawberry [173], and to delay ripening and decay of avocado [174], mango [175, 176], apple [177], pear [178], peach [179], strawberry [173], and potato [34]. The reasons for these benefits range from alleviating disorders resulting from calcium deficiency, to the effect of calcium on cell walls that makes them more resistant to decay [177] and firmer in texture, to adversely affecting conidial germination and germ-tube elongation [180]. The mineral salt $CaC1_2$ is considered GRAS [95]. There is a new compound called Nature Seal that claims to contain vitamins and minerals, likely calcium ascorbate. This product is used to control browning of cut fruits and vegetables like cut apple [181, 182].

33.5.5.2 Growth Regulators

The polyamines putrescine and spermidine altered texture when infiltrated into apples [32], and spermine and spermidine increased firmness in sliced strawberries [183]. Growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were added to fruit wax as antisenescent compounds to extend the shelf life of mandarin oranges [184]. Maleic hydrazide (250 ppm) and 2,4,-D were added to wax emulsions to delay ripening of mango fruits [185]. Gibberellic acid (150 ppm) suppressed sprouting of yam tubers for one month [186]. The only approved postharvest growth regulator treatment for fruits in the United States is for lemons destined for long-term storage. These fruit are sometimes treated with 2,4,-D to delay senescence of the button (calyx and residual stem) to reduce infection from *Alternaria* [187].

33.6 FUMIGATION AND GAS TREATMENTS

Hydrogen peroxide is an antibacterial agent based on its oxidative properties [36], effective at concentration ranges of 0.01–0.1% (U.S. FDA 21 CFR 184.1366) [95]. It is especially effective against gram-negative bacteria such as coliforms. There is little information as to its effect on fungi. It is mostly used to extend the shelf life of dairy products and is considered GRAS as an antimicrobial agent for treatment of milk for the making of cheese, whey, and starch [95]. It is reportedly effective as a vapor phase treatment of fumigant for fresh table grapes. Previously, fumigation with sulfur dioxide was used, but there are concerns about adverse effects on some sensitive individuals (U.S. FDA 21 CFR 182.3862) [95, 188]. Acetaldehyde vapor (0.25–0.5%) significantly reduced decay of harvested table grapes [189], while acetic acid vapor fumigation reduced fungal decay of grapes, apples, oranges, tomatoes, and strawberries [190, 191]. Other natural fruit and plant volatiles have been found to exhibit fungistatic activity. Of these volatiles, benzaldehyde, methyl salicylate and ethyl benzoate, 1-hexanol, trans-2-hexenal 2-nonanone, and furan compounds were found to be particularly effective [192–195]. The antimicrobial activity of CO_2 is greatest against molds and gram-negative psychrotrophic bacteria in the concentration range of 10–100% (U.S. FDA 21 CFR 184.1240) [36, 95]. The mechanism of action is not known but may be related to lack of O_2, acidification of intracellular contents, or effect on enzymes. Various concentrations of CO_2 used in modified-atmosphere packaging (MAP) are often within the microbistatic range [196, 197]. It has been shown to reduce brown rot of package cherry fruit [198]. 1-methylcyclopropene (1-MCP) is an ethylene action inhibitor, and thus delays changes caused by the plant hormone ethylene such as ripening, softening, and undesirable color changes such as browning in cut apples [182, 199]. It is currently used by the apple industry to prolong storage life of whole apples and is commercially sold under the name of Smartfresh. However, as ethylene promotes volatile synthesis, use of 1-MCP, by inhibiting ethylene action, can reduce aroma volatiles [200].

33.7 FRUIT QUARANTINE TREATMENTS

Fruit flies are major pests worldwide, and their fruit hosts must be treated to kill 100% of the immatures inside the fruits prior to export to uninfested areas of the world. One of the main treatments currently used is methyl bromide fumigation, which is scheduled to be phased out over the next few years since it is a suspected stratospheric ozone depleter [201]. Other treatments include cold storage, hot air, vapor heat, and hot water treatments [202]. Unfortunately, most of these treatments impart surface or internal quality damage to many fruits. Recently, the use of CA and edible coatings have been investigated as alternative treatments alone or in combination with currently used methods [203–205]. Preliminary findings suggest that lowered O_2 and elevated CO_2 may contribute to fruit fly mortality [204, 205]. Fruit coatings are already approved as a disinfestation treatment for surface mites on cherimoyas and limes from South America [206].

33.8 SURFACE PREPARATION AND COATING TECHNIQUES

Edible coatings can be applied by dipping products in coating materials and then allowing excess coating to drain as it dries and solidifies [64]. This was first reported for the Florida citrus industry [8] where the fruits were submerged into a tank of emulsion coating. Commodities are then generally conveyed to a drier where water or solvent is removed, or coated items can be allowed to air-dry under ambient conditions [207]. Sometimes a porous basket can be used to drain excess coating. Some emulsion coatings are applied with a foam applicator where a foaming agent is added to the coating or compressed air is blown into the applicator tank. The agitated foam is applied to commodities as they move by on rollers and cloth flaps or brushes distribute the emulsion over the surface of the commodity [207]. Excess coating is then removed by squeegees and sometimes recirculated. This type of emulsion contains little water and, therefore, dries quite quickly, but inadequate coverage is often a problem. Edible coating can be sprayed, which is especially useful to obtain a thinner and more uniform coat or if only one part or side of a product is to be coated [64]. This is the most popular method for coating whole fruits and vegetables, especially with the development of high-pressure spray applicators and air-atomizing systems. Overhead drip emitters can also deliver coating to fruit and

brushes below. The fruit tumble over rotating brush beds that become saturated with coating from overhead spray or drip applicators. As the fruit tumble and rotate over the saturated brushes they become uniformly covered with coating [207]. A controlled drop applicator allows drops of coating to be shattered in microdrops through a spray. Free-standing films can be cast such that the thickness can be controlled by spreading or pouring. A spreader with adjustable height can be used to control the thickness of the film, which is then allowed to dry [64]. Pan coating of tablets and candies involves a pan, which is enclosed and perforated along the side panels. The coating is delivered by a pump to a spray gun(s) mounted in various parts of the pan. The coating is atomized by the spray guns [207]. Solutions of antioxidant, preservatives, or other aqueous materials can be applied by dip or spray. In most cases, fruit and vegetable products are sanitized with chlorine and/or o-phenylphenate (SOPP) and dried on brushes or with fans as much as possible prior to coating. Sanitizing solutions are covered in U.S. FDA 21 CFR 178.1010 [95].

Coating of breakfast cereal products usually involves sugar-based materials, which presents a difficult situation since sugar is hydrophilic. With minimal water as a solvent, sugar is heated to a hard candy condition and sprayed on cereal, which is further heated to fuse the sugar. Otherwise, sugar is spun into a blanket on which cereal pieces are placed prior to the application of a second blanket of sugar. The whole sugar/cereal sandwich is then compressed and dried [16].

33.9 REPORTED APPLICATIONS IN FOODS

33.9.1 Fresh Intact Fruits and Vegetables

Edible coatings have been applied to a diverse array of whole fruits and vegetables since the 1930s and 1940s [15]. Coatings of vegetable or mineral oil have been applied to tropical fruits (mango, pineapple, banana, papaya, guava, and avocado) with varying degrees of benefit in terms of extending shelf life [208–211]. Sometimes oils, such as mineral oil, are used alone to coat fruits such as tomatoes or limes, but in such cases, they remain in a liquid state as a thin film on the surface of the fruit. The purpose of coating these fruits with oils is to help them slip over equipment (lubricant), add a slight sheen, delay ripening, delay weight (water) loss, and delay yellowing in the case of limes [15, 86, 212, 213]. Use of a cellulose-based film reduced the number of viable *Salmonella montevideo* cells on the surface of tomatoes in addition to retarding the ripening process [214]. Casein-lipid coatings reduced moisture loss from citrus, apple, and zucchini [65]. Shellac, carnauba wax, and polyethylene wax retard moisture loss and add shine to apples, citrus, and other fruit [15]. Waxing of potatoes with paraffin wax did not adversely affect respiration, but it did reduce sprouting and synthesis of chlorophyll (green pigment) and solanine (toxic glycoalkaloid) [215]. The Tweens lecithin and hydroxylated lecithin surfactants, and applied films were also useful in inhibiting chlorophyll and solanine synthesis in the peel of potato tubers [216, 217]. Creation of a modified atmosphere within the coated potatoes may have inhibited greening due to an effect on the synthesis of these undesirable compounds. In contrast, lipid and hydrocolloid coatings inhibited the degreening of lemons and limes [213] probably by inhibiting chlorophyll breakdown due to a modified atmosphere. The modified atmosphere induced by coatings can also be useful in retarding ripening as demonstrated with zein or chitosan on tomato [93, 133] and cellulose on mango and tomato [169].

33.9.2 Lightly Processed Fruits and Vegetables, Dried Fruit, and Nut Products

Coating of lightly processed fruits and vegetables is a new field [79, 85, 218], whereas coatings have been reported on nut and dried fruit products since the 1940s and 1950s [219]. Casein-lipid coatings reduced moisture loss [65], and a cellulose coating inhibited surface drying and the resulting color change (whitening) of peeled carrots [47, 220, 221]. There have been several reports of coatings applied to cut apple. Dextrin coatings prevented oxidative browning of apple slices [137], while a cellulose coating with antioxidants reduced cut apple discoloration more effectively than a solution of antioxidants alone [41]. A chitosan/lauric acid coating inhibited browning of cut apple [222], a caseinate/lipid coating reduced moisture loss [70], and an alginic acid/casein–lipid coating reduced water loss and browning of cut apple [79]. A bilayer coating of polysaccharide and lipid decreased water loss, respiration, and ethylene production in cut apple [223]. Pectin coatings were used to coat almonds and reportedly held salt and antioxidants on the surface while providing a nonoily texture [39]. Coating nuts with hydrogenated oils or acetylated monoglyceride containing an antioxidant increased their shelf life by retarding development of oxidative rancidity [40, 224]. Gum arabic has also been used to coat pecans [146]. Starch (amylose) and whey protein isolate coatings were used to reduce oxidative rancidity during storage of certain products such as nuts, cereal, beans [224–227], candies, and dried fruits [224]. This can also be controlled with antioxidants such as tertiary butylhydroquinone (TBHQ) alone or in a coating, as was shown for peanuts [40]. For coated candied fruit and dates, pectin coatings reduced stickiness [39]. Acetylated monoglycerides, hydrogenated coconut oil, or confectioner's butter stabilized dried fruit pieces in cake or bread mix [228] and raisins in cereals [229, 230]. Mineral oil, beeswax, vegetable oils, and acetylated monoglycerides have also been used to reduce clumping and stickiness of raisin products [229–233]. Vegetable oil blends as coatings for dried fruit have poor flavor stability [234]. Two commercial products, Spraygum® and Sealgum® (Colloides Naturels, Inc., Bridgewater, New Jersey), are based on gum acacia and gelatin. These products have been used to coat chocolates, nuts, cheese, and pharmaceutical products. In addition, they reduced the darkening of potatoes in combination with $CaC1_2$ [34].

33.9.3 Processed Food and Animal Products

Edible coatings are also applied to processed foods to restrict the movement of moisture and gases, especially O_2. Edible coatings can be used to prevent moisture loss and absorption

or transfer between components of differing water activity [16]. Edible coatings protect some commodities, such as meat and nut products, from oxidative rancidity, fat absorption, breading loss during frying [235, 236], and oil migration from such products as chocolate using hydrocolloids [237]. Amylose coatings provided a nonsticky surface at RH <80%, prevented fat migration from cheese and chocolate products, and retained volatile flavors [224]. Nonedible wax coatings are used to encase some cheese products [89, 238]. Meat fats, vegetable oils, and acetylated monoglycerides; mono-, di-, and triglycerides; waxes; and mixtures of these components have been used to coat meats and meat products including frozen chicken pieces and pork chops [235, 239]. These coatings protected the meat products from dehydration [16]. Cornstarch-alginate coatings reduced moisture loss and improved the juiciness of coated meat products [136], while HPC films reduced moisture loss from chicken [240]. Gelatin coatings have been used to coat or wrap meats [111] and form soft capsules in the pharmaceutical industry. Casein-lipid coatings reduced moisture loss from chicken eggs [65]. Emulsions of oil, water, and sugar have been used to coat cereal products to limit the entrance of water and therefore the amount of drying required [241]. Candies are sometimes coated with chocolate for flavor and reduction of moisture loss [242] or carnauba wax to add shine and reduce stickiness [16]. Corn zein and the derivatives MC and HPMC are oil repellent, and reduced oil absorption for potato balls or meat when included in batters or when applied as coatings [123, 240, 243]. These compounds also improved adhesion of batters or coatings to food products [122]. Coatings can delay the uptake of liquid by dry cereal products so that they last longer in milk before becoming soggy [225]. Such coatings often have a sugar base to impart a sweet flavor [16].

Heterogeneous products of differing water activities, such as ice cream cones with ice cream, or frozen filled pies where a dry material is in contact with a moist filling, have shown benefit from a barrier film between the two components. Such films had a polysaccharide component in a bilayer system with palmitic/stearic acid or saturated C_{16} and C_{18} fatty acids and beeswax [72, 74]. For example, a lipid-cellulose coating showed promise as a barrier to internal moisture migration between two components of a bread and tomato sauce product [71, 72].

33.10 LEGAL ASPECTS

Discussion of the various coating components discussed thus far in this review reflects rulings by the U.S. FDA. The United States allows coating of fresh produce with restrictions as to what can be used as a coating material or ingredient and, in some cases, the type of produce (usually dependent on whether the coated fruit or vegetable peel is normally consumed or not, i.e., apple versus avocado). Other countries, however, do not allow the coating of fruits and vegetables at all. For example, a ban has been reported in Norway on imports of waxed fruit. The Norwegian policy is that foods making significant nutritional contributions to the diet should be as free from additives as possible [244]. The German government tried to ban waxed apples, but the European Commission overruled it with a directive that allows apples, pears, and some other products to be imported from countries where waxing or coating of fruit is legal as long as the formulations contain beeswax, candelilla wax, carnauba wax, and/or shellac. Japan accepts shellac- and carnauba wax-coated citrus from the United States, but will not accept fruit coated with petroleum-based waxes that are legal in the United States for certain fruit, including citrus. The use of the cellulose derivative HPC is allowed in coating formulations in the United States, but not in New Zealand. Other countries such as Thailand and Australia are evaluating the applicability and acceptability of various coating film formers and additives [245, 246].

The U.S. FDA ruled in 1993 that all waxed fruit must be subjected to ingredient labeling regulations at the retail level [247]. Retailers are permitted to use collective names for coating ingredients, however, they should be prominently visible in the retail area where the commodity is displayed. The label or sign should indicate that the commodity is waxed or coated with food-grade animal-, vegetable-, petroleum-, beeswax-, and or shellac-based wax or resin to maintain freshness [246]. The United Kingdom proposed a similar labeling scheme requiring labels indicating all postharvest treatments applied to fresh produce [247]. Processed foods in the United States are required to contain a label on the package listing all additives. Coatings, preservatives, and antioxidants are all considered to be additives and, therefore, must be listed as individual ingredients. Postharvest use of fungicides, however, is not required to be labeled in the United States.

33.11 CONSUMER ATTITUDES

Consumers are becoming increasingly educated about health and nutrition, and are concerned about what goes on or into their food. Some consumer groups are concerned with the waxes and coatings themselves, and others fear that, in the case of fresh produce, harmful pesticide residues may be sealed inside the fruits by the coatings. There is also the issue of the use of animal products in coatings. Although there is a federal law in the United States about labeling coated produce, it is not always enforced. This presents a problem for Muslims, vegetarians, and Orthodox Jews, who have concerns about animal-derived products, and for those with certain protein allergies and intolerances. Enforcement of the regulations described in the previous section requiring a conspicuous sign would allow consumers to make an informed choice. Nevertheless, the main reason for coating produce such as apples and citrus is cosmetic. The fact remains that coated (shiny) fruits sell better than uncoated ones [248]. Some consumer groups want postharvest fungicide treatments to be listed as well as coating types [249].

33.12 CONCLUSION

In conclusion, edible coatings alone or as carriers of useful additives serve many functions for all types of food products.

They improve the external and internal quality characteristics of diverse commodities. Coatings can reduce dehydration and oxidation as well as the resulting undesirable changes in color, flavor, and texture. Waxes and other coatings delay ripening and senescence of fresh produce and can increase the microbial stability of lightly processed fruits, vegetables, and some processed products. Coatings show promise as environmentally friendly quarantine treatments. Most coating materials are produced from renewable, edible resources and can even be manufactured from waste products that represent disposal problems for other industries.

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34 Food and Bioactive Encapsulation

Fereidoon Shahidi, Priyatharini Ambigaipalan, Abrehem Abad, and Ronald B. Pegg

CONTENTS

34.1 INTRODUCTION

Encapsulation is an important process for the protection of food components and is widely applicable in all food sectors. At present, encapsulation is of growing interest in food processing and preservation. Various encapsulation techniques (spray drying, spray cooling, freeze drying, electrohydrodynamic techniques, fluidized bed coating, extrusion, coacervation, liposome, and emulsification) address some of the challenges to incorporating food ingredients, antioxidants, lipids, proteins, carbohydrate, minerals, vitamins, and bioactive compounds in powder form in food products. Encapsulation achieves excellent preservation, stabilization, and controlled release of bioactive compounds. Controlled release of bioactive ingredients at the right time and right place could also be provided by encapsulation. Encapsulation improves the effectiveness of food additives and enhances the shelf life of food, in addition to lowering the cost of the food products.

Ingredients are incorporated into the foods we eat for a multitude of reasons. For example, antimicrobial agents are added in an effort to ward off the early onset of microbial growth, antioxidants are used to prolong the shelf life of lipid-containing foods by protecting triacylglycerols and phospholipids against oxidative degradation, flavoring and coloring agents are added for the purpose of enhancing the sensory characteristics of the food, while various carbohydrate-based additives are employed to improve the rheological and textural properties of the product in question [1]. Encapsulation technologies have been increasingly attracting the attention of food ingredient suppliers and food product manufacturers as a means of achieving brand differentiation and enhancing product value. Microencapsulated products can add that extra zing, mask the taste of nutrients, alleviate processing problems, and increase the shelf life of food products [2]. Within the past decade, the explosion of functional foods and nutraceuticals globally and particularly in the North American market has in effect created a new type of additive for foods. These functional food ingredients, that provide a physiological benefit and/or reduce the risk of chronic disease beyond their basic nutritional functions, have led to the development of innovative product lines and functional food products. The ever-increasing health-conscious consumer is very much interested in the beneficial properties of functional food ingredients and nutraceuticals. Hence, the encapsulation (i.e., microencapsulation and nanoencapsulation) and controlled-release technologies, as a means to better deliver bioactives and nutrients in both wet and dry

applications, has grown both in scope and potential. Presently, there is a market interest in omega-3 fatty acids, phytosterols/stanols for cholesterol control, probiotics, prebiotics and synbiotics for gut health, vitamins and minerals for fortification/augmentation of food, bioactive peptides for controlling high blood pressure, amino acids and proteins as key ingredients in sports and energy foods and drinks, polyphenolic antioxidants that are promoted for general health benefits, and a number of lecithin products that are marketed for brain and memory function as well as liver support and cholesterol control [3]. According to the analyst from Frost and Sullivan's Research Service [4], "the growing functional food market is the major driving force behind microencapsulation innovation. Microencapsulation offers food companies a viable means of penetrating this lucrative growth sector because it has the ability to mask the taste associated with some of these ingredients.

Bioactive food components are prone to degradation or inactivation. Numerous bioactive food components can benefit from encapsulation due to protection and/ or prevention of their degradation until the product is delivered to the desired sites. These bioactive components include antioxidants, lipids, fatty acids, peptides, minerals, and vitamins but also live cells such as probiotics [5–8]. Many encapsulation procedures have been proposed but none could be considered as the universal one for all bioactive food components. This is caused by the fact that individual bioactive food components have their own characteristic molecular structures [9]. Nevertheless, they show extraordinary contrasts in solubility, molecular weight, and polarity, among others, which suggest that different encapsulation approaches must be applied in order to meet the specific molecular and physicochemical requirements for a specific bioactive component [9, 10]. Moreover, the compatibility of the bioactives is by all accounts not the only prerequisite an encapsulation procedure needs, but it ought to have characteristics to withstand environmental impacts [9]. The majority of bioactive compounds that have positive effects on human well-being are mostly derived from animal sources and the plant kingdom [11]. Because of the wide availability of encapsulated ingredients, many food products or functional food ingredients, whose development was thought to be technically difficult, are now possible. Such ingredients are products of a process in which the active ingredient is enveloped in a coating or "capsule," thereby conferring many useful properties to or eliminating undesirable properties from the original ingredient.

The real obstacle to the efficacious delivery of bioactive food components is not just hazardous events that occur during passage through the gastrointestinal tract, but the injurious circumstances during storage of the product that may affect the vehicle used for the bioactive components. It is therefore imperative to protect the bioactive component by using encapsulation procedures during the storage, transport, and the entire period of processing [12]. The most important requirement is that the encapsulation system protects the bioactive component from chemical degradation such as hydrolysis and oxidation to keep them fully functional [13]. Protection and controlled delivery of bioactive compounds at the right place and the right time can be achieved by encapsulation [14]. This chapter will focus on the types of bioactive components and highlight the challenges to delivery of bioactives, followed by a detailed explanation for the majority of materials used for encapsulation of food. Subsequently, it discusses different encapsulation techniques. Finally, the use of encapsulation in food applications is considered.

34.1.1 Purpose of Encapsulation

Encapsulation is a procedure that entraps one material (active agent) into another (wall material) in order to create particles in the micrometer (microencapsulation), millimeter, or nanometer (nanoencapsulation) scale [15, 16]. The encapsulated material (packaged) may also be called the fill, active core, payload phase, or internal material. On the other hand, the substance that encapsulates the active agent (packaging) is known as the coating, shell, membrane, capsule, external phase, carrier material, or matrix, and can be made of proteins, sugars, gums, natural and modified polysaccharides, synthetic polymers, or lipids [12, 17, 18]. Encapsulation technology has been used in food processing to provide an effective barrier against natural environmental parameters such as light, oxygen, and free radicals, among others [19]. Several types of encapsulants (matrix, reservoir, and coated matrix) have been characterized (Figure 34.1) as specified by Ray, Raychaudhuri, and Chakraborty [20].

The science of encapsulation deals with the manufacture, analytical evaluation, and application of encapsulated products. Despite the passage of time, the technology that has been developed for the food industry remains relatively unsophisticated compared to many other fields of application. This is a consequence of the limitations imposed on the food industry for the use of edible, low-cost ingredients and processing. Nevertheless, due to health-conscious consumers and the fashionable trend toward functional foods and nutraceuticals/natural health products, it is only now that manufacturers have been exploring new encapsulation delivery methods to ensure the bioavailability of efficacious quantities of desired functional food ingredients. Nanotechnology is one of the recent developments in which, for example, liquid designed for clear beverages, using small particle size nanocapsules has been considered. In nanoencapsulation, products can be reduced to a size of 30 nm and encapsulated in food compounds; the process is essentially self-assembly once the base encapsulation

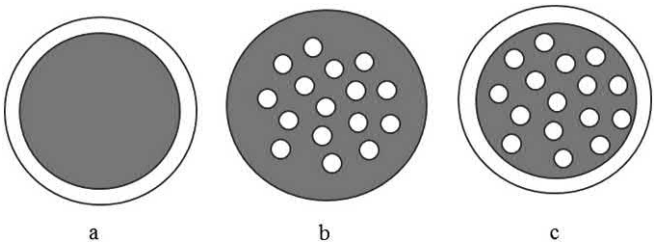

FIGURE 34.1 Types of encapsulates: (a) reservoir, (b) matrix, (c) coated matrix. (From Ray et al. [20].)

material is finalized. In effect, nanotechnology provides an improved activity level and solubilized ingredients, for example, for clear beverages, that previously could not be used.

King [21] notes that it is important for food scientists to distinguish between encapsulation versus entrapment of food ingredients. He states that encapsulation may be defined as a process of forming a continuous thin coating around encapsulants (i.e., solid particles, droplets of liquids or gas cells), which are wholly contained within the capsule wall as a core of encapsulated material. On the other hand, entrapment refers to the trapping of encapsulants within or throughout a matrix (e.g., gel, crystal), but a small percentage of the entrapped ingredients will normally be exposed at the particle surface, whereas this would not be so for the encapsulated product. As mentioned, the entrapped material is commonly a liquid but could be a solid particle or gas, and is referred to by various names such as core material, payload, actives, fill, or internal phase [22]. The material that forms the coating is referred to as the wall material, capsule, membrane, carrier, shell, or coating.

The food industry applies encapsulation for a number of reasons [23–25]:

(i) Encapsulation/entrapment can protect the core material from degradation by reducing its reactivity toward the outside environment (e.g., heat, moisture, air, and light).
(ii) Evaporation or transfer rate of the core material to the outside environment is decreased/retarded.
(iii) The physical characteristics of the original material can be modified and made easier to handle. For example, (a) a liquid component can be converted into solid particles; (b) lumping can be prevented; (c) the core material can be distributed more uniformly throughout a mix by giving it a size and outside surface; (d) hygroscopicity can be reduced; (e) flow and compression properties can be improved; (f) dustiness can be reduced; and (g) density can be modified.
(iv) The product can be designed to either release slowly over time or to release at a certain point (i.e., to control the release of the core material so as to achieve the proper delay until the right stimulus).
(v) The taste of the core material can be masked.
(vi) Encapsulation can dilute the core material when it is needed only in very small amounts, but to achieve uniform dispersion in the host material.
(vii) Encapsulation can be used to separate reactive components within a mixture, which would react with one another.

34.1.2 Challenges to Delivery of Bioactive Compounds

Most food bioactives, especially lipids, have very low water-solubility. For example, ω-3 oils, oil-soluble vitamins, phytosterols, and the like are difficult to directly incorporate in aqueous-based foods, such as sauces, beverages, yogurts, desserts, and dressings. Instead, they should be incorporated into delivery systems via encapsulation or emulsification [26]. Some bioactive lipids, such as carotenoids and phytosterols as well as the compound curcumin, are crystalline at room temperature and therefore difficult to be directly incorporated into foods. This is because they tend to form sediments or give an unpleasant appearance that might be overcome by using high temperatures; however, heating may adversely impact the stability or sensory properties of products [8, 27].

A number of important lipophilic bioactives such as carotenoids, curcumin, and ω-3 oils are prone to chemical degradation during storage or after ingestion [28–31]. The rate and the extent of chemical degradation depend on the nature of the bioactive compound being considered and environmental conditions such as temperature, pH, oxygen, light, and the presence of prooxidants. Therefore, it is necessary to develop delivery systems that can protect these bioactives from chemical degradation [8, 26]. Many of the lipophilic bioactive components have variable and/or low bioavailability within the human gastrointestinal tract. This might occur for a number of reasons, including transformation in the gastrointestinal fluids, poor absorption, or low bioaccessibility [26, 32]. In this case, nanoscale delivery systems might be prepared in order to modulate these processes and to increase bioavailability [26]. Some of the lipophilic bioactives might physically interact with other components in the system leading to instability. Therefore, it might be necessary to use a nanoscale delivery system to isolate one component from others in the system in order to make them unavailable for interaction with each other [26]. Furthermore, lipids must maintain their bioactivity within the human body before being delivered to the desired site. For instance, they might have to resist enzyme activity and high acidity of the stomach [8].

Bioactive proteins are also quite sensitive to degradation. These proteins have to reach the small bowel in an intact conformation in order to exert a beneficial health impact and to inhibit their biodegradation in the gastrointestinal tract. However, it must not always be interpreted that all food proteins should be encapsulated. Most of the proteins and peptides also require hydrolysis in the small intestine and stomach in order to release specific amino acids or bioactive peptides [7, 8, 33, 34].

In the case of bioactive carbohydrates, the main classes that need to be delivered into foods are dietary fibers. Soluble, nondigestible carbohydrates isolated from their natural environment and converted to food ingredients are added into food products, instead of fibers that are naturally present in whole foods, such as fruits, vegetables, and grains. This is because some isolated soluble fibers are bioactive food components and, as such, might need to be encapsulated in delivery systems. A well-designed structured delivery system might be capable of increasing the total amount of dietary fiber in a food without impacting its physicochemical or organoleptic properties [8].

Probiotic bacteria are at present the driving force in the design of functional foods and maintaining their functional impact for supporting health. Probiotic bacteria are defined as live microorganisms that when administered in sufficient quantities confer a beneficial physiological impact in the host,

as is their use in dairy products [13]. Application of encapsulation to living probiotics is limited; the size of probiotics (1–5 μm) excludes some of their nanotechnological use in encapsulation. Hence, we usually refer to microencapsulation, rather than nanoencapsulation, when discussing the encapsulation of probiotics [7, 8, 10]. Microencapsulation of many types of probiotics might be mandatory for achieving their promised health benefits [8]. Though several health benefits of probiotics in dairy products have been described, it is not easy to allow long-term survival of probiotics in traditional dairy and fermented products [35]. Probiotics are influenced by several factors such as difference in pH including the postacidification in some fermented products [10, 35]. In addition, the oxygen toxicity during processing and packaging has been shown to be detrimental for the survival of probiotics [10].

34.1.3 Benefits and Types of Microcapsules

Microencapsulation is defined as the technology of packaging solids, liquids, or gaseous materials into miniature, sealed capsules that can release their contents at controlled rates under specific conditions [4, 36, 37]. The small packages, called "microcapsules," may range from submicron to several millimeters in size and have a multitude of different shapes, depending on the materials and methods employed to prepare them. Generally speaking, the microcapsule has the ability to modify and improve the apparent shape and properties of a substance. More specifically, the microcapsule has the capacity to preserve a substance in the finely divided state and to release it when the occasion demands.

Microcapsules offer the food processor a means to protect sensitive food components, ensure against nutritional/nutrient loss of functional food ingredients, utilize otherwise sensitive food-grade materials, incorporate unusual or time-release mechanisms to a formulation, mask or preserve flavors and aromas, and transform liquids into easily handleable solid ingredients [38]. The unusual properties afforded by encapsulated ingredients offer the food technologist greater flexibility and control in developing functional foods that are more flavorful, nutritious, and may offer physiological benefits, such as a reduction in the likelihood of developing a chronic disease, beyond basic nutritional functions, in order to meet the expectations of today's health-conscious consumers. Microencapsulation technology, however, is sometimes considered more of an art than science. In *Microcapsule Processing and Technology*, Asajo Kondo [39] states, "Microencapsulation is like the work of a clothing designer. He selects the pattern, cuts the cloth, and sews the garment in due consideration of the desires and age of his customer, plus the locale and climate where the garment is to be worn. By analogy, in microencapsulation, capsules are designed and prepared to meet all the requirements in due consideration of the core material, intended use of the product, and the environment of storage."

Various properties of microcapsules may be changed to suit specific ingredient applications, and these include composition, mechanism of release, particle size, final physical form, cost, and regulations. Before considering the properties desired in encapsulated products, the purpose of encapsulation must be clear. In designing the encapsulation process, the following questions should be taken into consideration: (i) What functionality should the encapsulated ingredients provide to the final product? (ii) What type of food-grade coating material should be selected? (iii) What processing conditions must the encapsulated ingredient survive before releasing its content? (iv) What is the optimum concentration of the active material in the microcapsule? (v) By what mechanism will the ingredient be released from the microcapsule? (vi) What are the particle size, density, and stability requirements for the encapsulated ingredient? (vii) What are the cost constraints of the encapsulated ingredient? (viii) Will the encapsulated ingredient meet regulations/standards to be considered as a functional food ingredient?

The architecture of microcapsules is generally divided into several arbitrary and overlapping classifications. One such classification is known as matrix encapsulation. This is the simplest structure in which a sphere is surrounded by a wall or membrane of uniform thickness, resembling that of a hen's egg. In this design, the core material is buried to varying depths inside the shell. This microcapsule has been termed as a single-particle structure. In addition to this structure, it is also possible to design microcapsules that have several distinct cores within the same capsule, or more commonly, numerous core particles embedded in a continuous matrix of wall material. This type of design is termed the aggregate structure. The particles in the aggregate structure need not be all of the same material. In the case of aggregate capsule structures, a degree of particle size control can be achieved. This technique has been accomplished with numerous materials to improve size distribution properties [40]. Another well-known design for a microcapsule is to form a multiwalled structure, in which the different concentric wall layers can have the same or quite different compositions. In this case, the multiple walls are placed around a core to achieve multiple purposes related to the manufacture of the capsules, their subsequent storage, and controlled release. This design is particularly important for controlled-release encapsulation systems using nanospheres containing an active ingredient. These nanospheres can be encapsulated with other ingredients, such as flavors, cooling or heating agents, or sweeteners, within a microsphere. Upon exposure to water or pH the microsphere releases its contents, and over an extended period of time the nanosphere releases the encapsulated active ingredient via molecular diffusion and possibly by enzymatic degradation. The surface properties of the nanospheres can be altered to be bioadhesive or charged negatively or positively depending on the intended target site [41].

The theory and application of microcapsular delivery systems encompass a variety of engineering techniques and scientific disciplines, thus making it difficult to present a systematic view of the total effort being dispensed in this field. This contribution summarizes the art of microencapsulation as it relates to the food industry and presents current information on the process of encapsulation. To accomplish this, a comprehensive examination of various encapsulating matrices currently utilized by the food industry is included. In

addition to their general description, the advantages and disadvantages they offer as an encapsulating agent when forming microcapsules is discussed. An in-depth examination of the various microencapsulation techniques follows. This includes the processes of spray drying, spray cooling and spray chilling, fluidized bed coating, extrusion, centrifugal extrusion, lyophilization, coacervation, centrifugal suspension separation, cocrystallization, liposome entrapment, interfacial polymerization, inclusion complexation, and nanoencapsulation. Afterward, encapsulated ingredients and their application to various food systems are considered with reference to some of their common uses and growing importance to the functional food and nutraceutical arena. Finally, an examination of what is meant by controlled release and some of the mechanisms surrounding it are discussed.

34.2 ENCAPSULATION MATRIX

In order to encapsulate a functional food ingredient, the first requirement is the selection of an appropriate coating material referred to as the encapsulating matrix. As indicated earlier, many researchers have referred to the coating material as the shell, wall material, or encapsulating agent [42]. Coating substances, which are basically film-forming materials, can be selected from a wide variety of natural or synthetic polymers, depending upon the material to be coated and the characteristics desired in the final microcapsules. It is the composition of the coating material that is the main determinant of the functional properties of the microcapsule and of how it may be used to improve the performance of a particular ingredient. An ideal coating material should have the following properties: (i) good rheological properties at high concentrations and be easy to work with during the process of encapsulation; (ii) a capability to disperse or emulsify the active material and to stabilize the emulsion so produced; (iii) nonreactivity with the material to be encapsulated both during processing and upon prolonged storage; (iv) a capability to seal and hold the active material within its structure during processing and storage; (v) a complete release of the solvent or other materials, which are used during the process of encapsulation, under drying, or other desolventization conditions; (vi) a capability to offer maximum protection to the active material against environmental conditions (e.g., oxygen, heat, light, and humidity); (vii) solubility in solvents acceptable by the food industry (e.g., water, ethanol); (viii) chemical nonreactivity with the active material; (ix) standards that meet specified or desired solubility properties of the capsules, and release properties of the active material from the capsule; and (x) low cost. Because no single coating material meets all of the aforementioned criteria, in practice, coating materials are employed either in combination or with modifiers such as oxygen scavengers, antioxidants, chelating agents, and surfactants. Many substances can be encapsulated such as liquids, solids, or gases of different origins, types, and properties. However, only a limited number of substances have been certified for food applications as generally recognized as safe (GRAS) materials. It is worth noting that the regulations for food additives are stricter than for pharmaceuticals or cosmetics. As a result, some compounds that are widely accepted for drug encapsulation are not approved for use in the food industry [43]. Some commonly used coating materials are presented in Table 34.1 and discussed in detail next.

TABLE 34.1
Coating Materials for Encapsulation of Food

Origin	Carbohydrate Polymer	Protein	Lipid
Plant	Starch – Derivatives (maltodextrins, corn syrup solids, dextran, modified starch, sucrose) Cellulose – Derivatives Plant extracts – Galactomannans – Soluble soybean Plant exudates – Gum arabic – Gum karaya – Mesquite gum Polysaccharide	Gluten (corn)	Fatty acids/ alcohols Glycerides Waxes Phospholipids
Microbial/ animal	Xanthan Dextran Gellan Chitosan	Whey proteins Caseins Gelatin	Fatty acids/ alcohols Waxes Glycerides Phospholipids
Marine	Alginate Carrageenan		

Sources: Pegg and Shahidi [1]; Wandrey et al. [43].

34.2.1 Carbohydrates

Carbohydrates comprise about 90% of the dry mass of all biomass and about 90% thereof are polysaccharide polymers. These natural and copolymers are composed of sugar derivatives and/or residues. Many of the native polysaccharides contain a small percentage of peptides and proteins remaining from their biosynthesis. However, these are generally removed during the processing [43]. The ability of carbohydrates to absorb and adsorb volatiles from the environment or to retain them tenaciously during the drying process has important implications and applications for flavor encapsulation. In fact, carbohydrates are the most commonly used coating material in flavor encapsulation processes.

The mechanisms by which carbohydrates retain volatiles during processing such as freeze drying and spray drying as well as extrusion are not fully understood but most probably involve physical interactions [44]. It has been postulated that the formation of microregions during freeze drying, which contain highly concentrated solutions of carbohydrate and volatiles, results in molecular association of the carbohydrate through hydrogen bonding. This in turn creates a stable network and traps the volatiles [45]. For example, it has been

reported that loss of volatiles from lactose during freeze drying increases when the material changes from an amorphous solid to a crystalline one [46]. Formation of cracks in the microregion structure might have accounted for this [44].

The two major processes used for encapsulation of food flavorings are spray drying and extrusion [47], both of which depend primarily on carbohydrates used for the encapsulation matrix [48]. While one can find examples of encapsulation of fats (e.g., spray chilling), proteins (gelatine), and inorganics (fused silica), carbohydrates constitute the majority of the market of encapsulation matrices. While many compounds are classified as carbohydrates, the discussion here does not include all such compounds. Some are discussed under other headings and classifications. Figure 34.2 presents the classification of natural food-grade materials with their origin and Figure 34.3 presents the relevant structural information [43].

34.2.1.1 Modified Starch

Starch, the reserve polysaccharide of most plants, is one of the most naturally abundant polymers found on earth. It is extracted from numerous food sources including tubers (potato, tapioca, arrowroot, and sweet potato) and cereals (corn, waxy maize, wheat, and rice). It is the most commonly used food hydrocolloid and this is partly because of the wide range of functional properties it can provide in its native and modified forms, and partly because of its low cost relative to alternatives [49].

Starch comprises polymers of glucose units linked together primarily by α-(1⟶4) bonds and secondarily by α-(1⟶6) bonds. In the native state, starch exists as granules that are insoluble in cold water due to hydrogen bonding of the polymer chains. The two polymer types found within the granules are amylose, a straight-chain polymer, and amylopectin, a branched-chain polymer. With its long, straight chains, amylose is known for forming strong, flexible films. On the other hand, due to its extensive branching, amylopectin is not a strong film former, but is noted for clarity and stability when forming gels and may show a slightly greater tendency toward absorption or binding of flavors. The content of amylose and amylopectin in starch granules varies depending upon the source: typically starch contains 18% to 30% amylose, except for waxy corn types, which are virtually all amylopectin [49]. When mixed with water and provided with enough heat, starch granules swell sufficiently as hydrogen bonds are broken to form pastes that can produce strong films. For most encapsulation processes, however, the viscosity of native starch is too high.

Starch presents an interesting situation with regard to flavor binding. Because the amylose fraction forms helical structures, starch can entrap flavor molecules, thereby producing

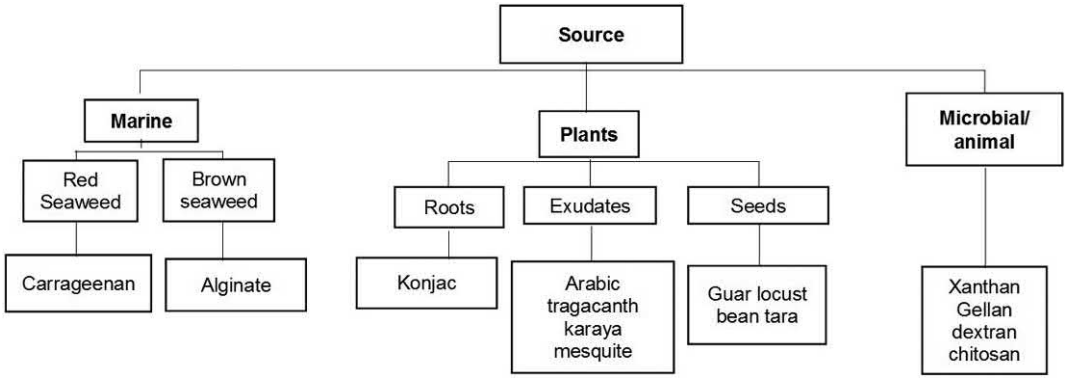

FIGURE 34.2 Classification of natural carbohydrate polymers.

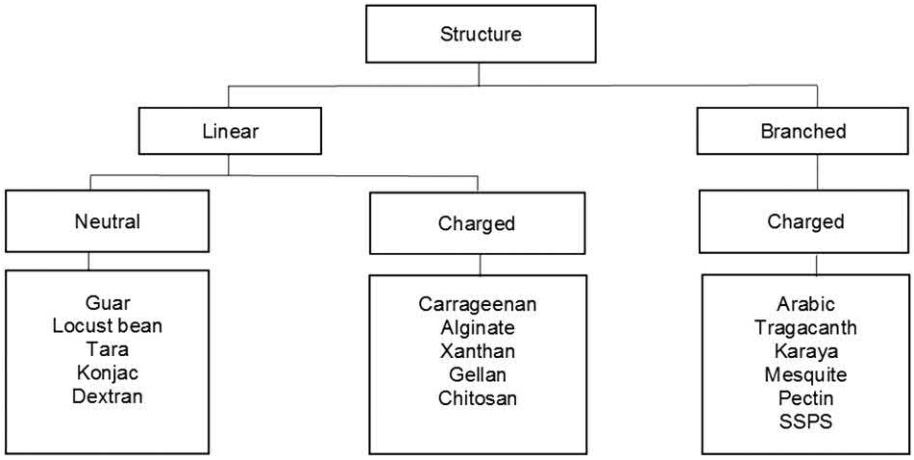

FIGURE 34.3 Classification of carbohydrate polymers related to structural characteristics. (From Wandrey et al. [43].)

very stable complexes [50–52]. However, starch is hydrophilic and hydrolysates derived from it afford virtually no emulsification properties to the compound being encapsulated.

Both physical and chemical modifications of starch are known. Many functional derivatives of starch are marketed, including oxidized, cross-linked, acetylated, partially hydrolyzed, and hydroxypropylated starch. The main reason for starch modification is to change the structure and impact the hydrogen bonding in a controllable manner in order to promote and extend the industrial applicability of such commodities [53]. Cross-linking replaces a few hydrogen bonds between chains in starch by covalent bonds, thus swelling is inhibited. The shelf life can be improved and retrogradation can be prevented. The starches modified in this way show better tolerance to temperature fluctuations [43]. In its natural state, starch is cold-water insoluble [49]. In dextrinization, starch is heated in the dry granular form with agitation, generally in the presence of an acid such as hydrochloric or sulphuric. The term *dextrinization* refers to the reduction in size of the starch molecule to smaller fractions or dextrins. Partial hydrolysis of starch granules ensues as well as repolymerization to form more highly branched polymers. The extent of this process can be varied based on time, temperature and pH conditions to yield products with different solubility and viscosity characteristics. Dextrins have increased cold-water solubility and lower solution viscosity than gelatinized native starch. If heated too long the products become darker and stronger reaction flavors are noted. Unfortunately, these strong color and flavor characteristics and a lack of lipophilic emulsifying qualities make dextrins less than ideal for encapsulation, especially for oil-based products. Dextrin formulations could be prepared at higher concentrations than the unmodified starch yielding films with higher proportions of solids that dry faster and are thicker. For example, yellow corn dextrin is used to encapsulate oils and water-insoluble flavors by spray drying [43].

The lack of emulsification properties of native starch creates two major problems. The first is that poor flavor retention results. The fineness of the infeed emulsion has a strong influence on determining the extent of flavor retention during drying. The second problem relates to the stability of the flavor emulsion once reconstituted in the final product. If the carrier provides no emulsification to the flavor, then the flavor rapidly separates from the product and forms a ring at the top. For a compound to function as an emulsifier, it must contain both lipophilic and hydrophilic groups. To overcome this problem, starches can be modified chemically to change their functional characteristics. For example, the US Food and Drug Administration has approved the reaction of starch with 1-octenylsuccinic anhydride to form a modified starch containing both hydrophobic and hydrophilic groups. The level of substitution, usually in the range of 0.02%, results in a product that is vastly different from that of the native starch. The addition of lipophilic moieties along the starch polymer permits the formation of emulsions with tight alignment of the polymer around an oil droplet. This stabilization is extremely important for encapsulation of lipids and lipid-containing products. Modified starch provides excellent retention of volatiles during spray drying and can be used at a higher infeed solids level than gum acacia (also known as gum arabic). While gum acacia is generally limited to use at about 35% level of infeed solids, modified starch can typically be used at levels approaching 50% [48]. The high solids level helps to reduce the loss of encapsulated ingredients and increases spray dryer throughput.

The emulsification properties of lipophilic starches, as well as oil retention in the spray dried powders, are reported to be equal to or greater than that of gum acacia [54, 55]. Modified starch also excels in promoting emulsion stability. One means of doing so is to produce small particle size droplets. Solutions of gum acacia produced an average emulsion droplet size of about 3 μm, and modified starch gave droplets of less than 2 μm. The emulsions made with modified starch were physically more stable than those made with the standard gum acacia [47]. Reineccius [48] pointed out some disadvantages of modified starches. For example, they are not considered natural for labeling purposes and often have an undesirable off-flavor and may not afford good protection to oxidizable flavorings.

34.2.1.2 Maltodextrins and Corn Syrup Solids

Maltodextrins, $(C_6H_{12}O_5)_nH_2O$, are nonsweet nutritive polysaccharides consisting of α-(1⟶β4)-linked D-glucose units. However, in order to be termed maltodextrin, they must possess a reducing sugar content or dextrose equivalence (DE) of less than 20. Maltodextrins are prepared as white powders or concentrated solutions by partial hydrolysis of corn starch with safe and suitable acids or enzymes. If the DE equals or exceeds 20, they are referred to as corn syrup solids. The DE, which is a percentage, is a measure of the reducing power of a sample compared to an equal weight of dextrose. Common designations of maltodextrins are 5, 10, 15, and 18 DE, while commercial corn syrup solids have 20, 25, 36, and 42 DE [56]. Products with a DE greater than 42 cannot be easily dried and hence are sold only as concentrates/syrups. Because maltodextrins and corn syrup solids are so closely related to one another in terms of their physical and chemical properties, as well as their applicability to food ingredient encapsulation, they will be discussed jointly. A flow diagram for the production of maltodextrins and corn syrup solids from corn starch is presented in Figure 34.4.

In the production of maltodextrins and corn syrup solids, starch is only partially hydrolyzed by acid or enzymes; thus, the resulting products are heterogeneous mixtures of various chain-length glucose polymers. The higher the DE, the higher the concentration of product that can be put into solution. In spray dried encapsulations, increased levels of soluble solids at a low viscosity is a major factor in the efficiency of production. In spray dried encapsulation of citrus oils, Anandaraman and Reineccius [57] reported that the higher the DE of the corn syrup solids used, the longer the stability of the encapsulated oil. Bangs and Reineccius [58] found intermediate or lower DE products to be more efficient for spray dried encapsulation of volatile artificial flavor compounds. It was postulated that a

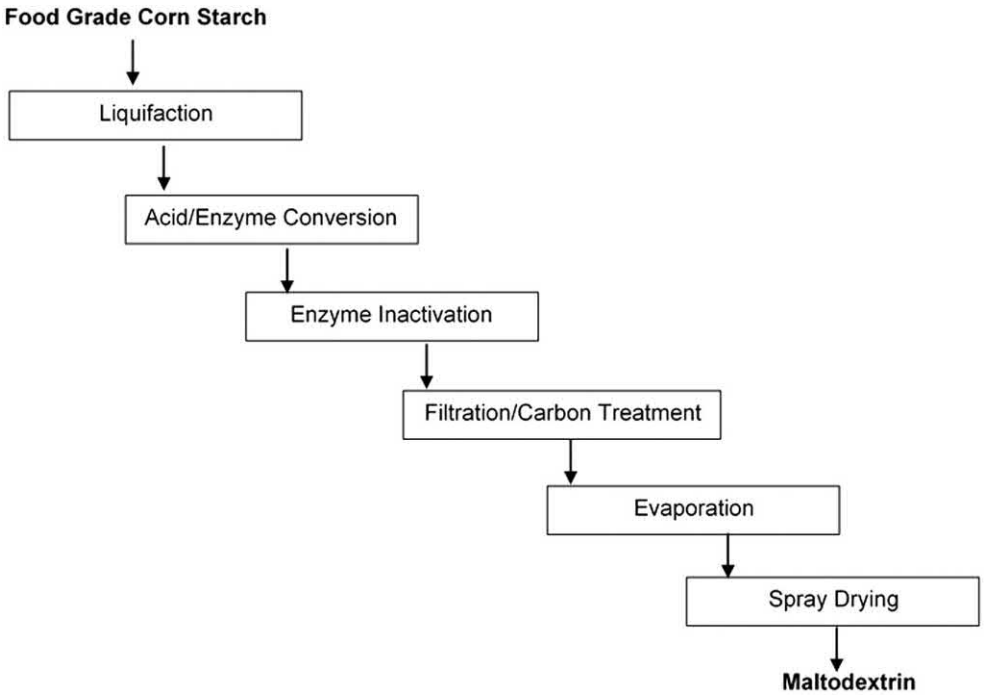

FIGURE 34.4 Flow diagram for the production of maltodextrin and corn syrup solids from corn starch.

balanced polymer length might aid in trapping the volatiles as the surface of the droplet dries.

These hydrolyzed starches offer the advantages of being relatively inexpensive (approximately one third that of modified starches), bland in flavor, and low in viscosity at high solids content. However, the major problem with these products is the lack of emulsification properties [59]. Since most active materials (especially the flavors) are insoluble in aqueous solutions and thus exist as emulsions, emulsion stability is viewed as an important consideration when selecting a coating material. Maltodextrins and corn syrup solids lack lipophilic characteristics and have virtually no emulsion-stabilizing effect on water-insoluble components [48]. It is also found that maltodextrins and corn syrup solids do not result in good retention of volatile compounds during the spray drying process. Corn syrup solids typically perform more poorly. Retention often ranges between 65% and 80% [48]. The retention capacity changes significantly with the difference of the DE values. Raja et al. [60] investigated the use of maltodextrins with varying DE values for encapsulating cardamom oil. The reason for the poor retention of volatiles by maltodextrins and corn syrup solids was believed to be due to their poor film-forming capabilities (*nota bene*, this is why they are sometimes referred to as carriers and not encapsulators). The wet encapsulation matrix must form a film around the droplets of active material in order to effectively retain them during the drying process and water removal. It is considered that since maltodextrins and corn syrup solids have no emulsification properties, they produce coarse emulsions and therefore result in poor flavor retention during drying [61].

Maltodextrins and corn syrup solids vary greatly in protecting encapsulated ingredients from oxidation. There is a strong dependence of associative stability on DE of the hydrolyzed starch. The encapsulated product with the highest DE is extremely stable and would have a shelf life of years without use of an antioxidant [57]. Several factors have been attributed to the outstanding protection afforded by high-DE coating materials. It has been considered that the higher-DE systems are less permeable to oxygen and therefore offer better protection to encapsulated ingredients [48]. One should also keep in mind that the presence of glucose in the encapsulation system has a considerable effect on the antioxidative properties.

34.2.1.3 Cyclodextrins

Cyclodextrins are chemically and physically stable molecules formed by enzymatic modification of starch. They have the ability to form complexes with a wide variety of organic compounds within their ringed structure, most notably flavor compounds [62]. The ability of these unusual molecules to form inclusion complexes, which can change the physical and chemical properties of guest molecules, offers a variety of potential uses to the food industry. Although cyclodextrins have been studied for a century, and their ability to form inclusion complexes has been recognized for at least 50 years, they were not utilized for food applications until the 1970s when Japan and Hungary began producing them commercially.

Cyclodextrins were discovered in 1891 when Villiers reported their appearance in rotting potatoes. In 1904, Schardinger characterized them as cyclic oligosaccharides and identified *Bacillus macerans* as the bacterium that produced cyclodextrin glycosyltransferase (CGTase), the enzyme responsible for the generation of cyclodextrins from starch. Because of Schardinger's studies, cyclodextrins were initially referred to as Schardinger dextrins, but of more significance

was the fact that his work set the direction for future research, pointing it toward a study of the structure of cyclodextrins and their commercial production. French [63] has provided a detailed history of the development of cyclodextrins up to 1956. Cyclodextrins are completely metabolized by colon microflora because they are not absorbed in the upper gastrointestinal tract [64].

Today, cyclodextrins are produced from starch by selected microorganisms such as *Bacillus macerans* and *Bacillus circulans*, which have CGTase activity. After cleavage of starch by the enzyme, the ends are joined to form circular entities with α-(1⟶4) linkages. Because cyclodextrins are closed circular molecules, glucoamylases and beta amylases cannot hydrolyze them, as there is no reducing end group, which is necessary to initiate hydrolysis. The cyclic dextrins formed contain six, seven, or eight glucose monomers; these are referred to as alpha-, beta-, and gamma-cyclodextrin, respectively. The glucose monomers are joined to one another in a doublenut-shaped ring, giving the cyclodextrin is a molecular structure that is relatively rigid and has a hollow cavity of specific diameter and volume. Depending upon the enzyme used and the conditions under which the reaction is performed, the ratio of cyclodextrins can vary from various mixtures to a single cyclodextrin being formed.

Figure 34.5 shows the chemical structure of α-, β-, and γ-cyclodextrins. β-Cyclodextrin is the predominant

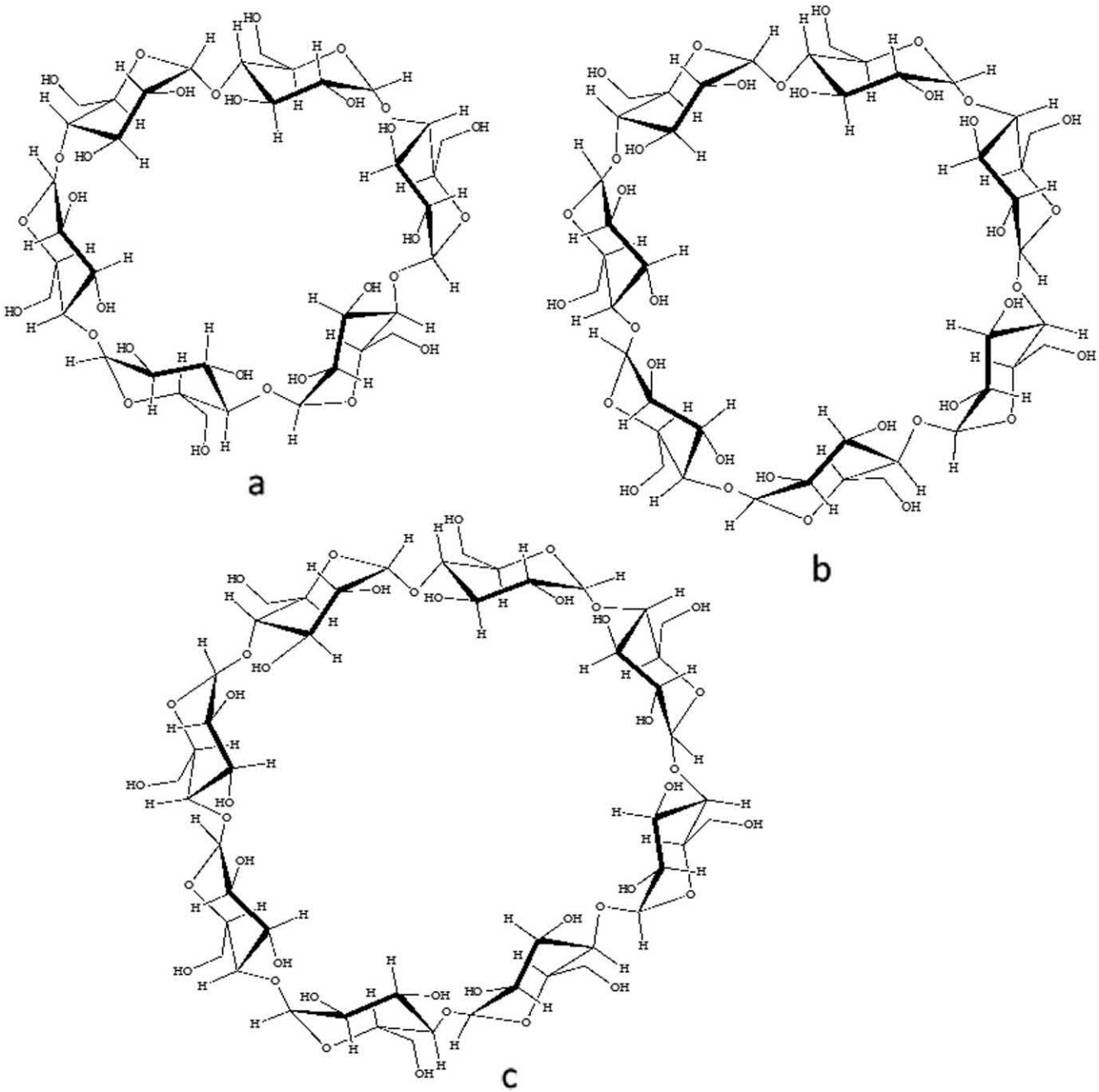

FIGURE 34.5 Chemical structures of (a) α-cyclodextrin, (b) β-cyclodextrin, and (c) γ-cyclodextrin.

cyclodextrin produced by CGTase enzymes. Polar hydroxyl groups of the glucose monomers are located on the rim of the molecule and are directed away from the cavity. These groups interact with water giving cyclodextrins their aqueous solubility properties and will interact with polar groups of some molecules to form hydrogen bonds. While the outer surfaces (top and bottom) are hydrophilic, the internal cavity has a relatively high electron density and is hydrophobic in nature due to the hydrogen and glycosidic oxygen atoms being oriented to the interior of the cavity.

Organic molecules of suitable size, shape, and hydrophobicity are able to interact noncovalently with cyclodextrins to form stable complexes. Several forces, such as van der Waals forces, hydrophobic interaction, and dipole–dipole interaction, are involved in the binding of guest molecules to the cyclodextrin cavity. These forces are sufficiently efficacious to form a stable complex but are not so secure that the guest molecule can be released from the complex to become available for the intended effect of the guest molecule [65].

The dimensions of the cyclodextrin's cavity allow some selectivity for the complexation of guest molecules. Strong binding results if more interaction occurs between the walls of the cyclodextrin and the guest molecule. If the molecule to be encapsulated is small compared to the cavity, only part of its surface is in contact with the walls and the full potential of the guest molecule to interact with the cyclodextrin is not realized. For molecules containing five or fewer carbon atoms, the smaller cavity of α-cyclodextrin affords more interaction between the molecule and the cavity walls. Better complexation results than if β- or γ- cyclodextrin were used. On the other hand, large bulky molecules, such as anthracene, fit into the cavity of the γ-cyclodextrin better than that of α- or β-cyclodextrin. In fact, sometimes, molecules are too large to fit into the cavity of one or more of these. Thus, the guest molecule might be totally excluded from the cavity, or only a portion of it would fit. As more of the molecule can fit into the cavity, a stronger binding results. Some of the physical properties of cyclodextrins are summarized in Table 34.2 [66].

β-Cyclodextrin deserves special attention because it is the most readily available cyclodextrin. In preliminary studies, it is generally used and is known to be able to form inclusion complexes with flavor ingredients of molecular masses ranging between 80 and 250 Da. Lindner [67] reported that the molecules of nearly all natural spices and flavors fit into this range. Much research has focused on the ability of cyclodextrins to prevent the volatilization of flavors and essences from spices, flavor extracts, and lipids. Nagatomo [68] reported that cyclodextrins improve the stability of spices for use in sausages and other meat products. Spices that have been included in cyclodextrins have demonstrated controlled flavor release. In addition, thermal stability is improved when fats are added to them. Nagatomo [68] also noted that cyclodextrins preserved the flavor of cookies, vegetable pastes, biscuits, citrus fruits, Japanese onions, garlic, celery, and a variety of other products. Pagington [69] reported that the strong odor of onion oil, garlic oil, and pyrazines was restricted by cyclodextrin use, but complexing with cyclodextrins prevented their flavor from being lessened in processing, and released their flavor directly into the mouth.

Natural pigments, such as carotenoids and anthocyanins, can also be stabilized by a cyclodextrin complex [68]. Pigments can be masked, or their color tones intensified. It has been reported that the colors can be changed through the inclusion complexation process. Cyclodextrin complexes can protect ingredients from oxidation, light-induced reactions, thermal decomposition, and evaporation loss. Crystalline complexes are stable and improve processing conditions, handling, and storage of food ingredients.

34.2.1.4 Modified Cyclodextrins

Although β-cyclodextrin forms a stable microcapsular structure, water solubility of β-cyclodextrin complexes is generally a problem. The solubility of α- and γ- cyclodextrin at room temperature is 12.8 and 25.6 g/100 mL water, respectively; whereas for β-cyclodextrin it is only 1.8 g/100 mL. As temperature increases, the solubility of cyclodextrins also increases, but their solubility can change when a guest is complexed. If the guest molecule is highly soluble in water, then the inclusion complex is more soluble than the cyclodextrin itself. The polar or ionic moiety of the guest molecule projects out of the cavity and contributes to the solubility of the complex along with the interaction of the hydroxyl groups of the cyclodextrin. On the other hand, complexation of the cyclodextrin with a guest molecule that is not soluble or only partially soluble in water generally results in a decrease in the solubility of the cyclodextrin. Although solubility of the complex is generally

TABLE 34.2
Physical Properties of Cyclodextrins

	Physical Properties						
			Molecular Dimensions (Å)				
Type of Cyclodextrin	Number of Glucose Units	Molecular Weight	Inside Diameter	Outside Diameter	Height	Solubility at 25°C (g/100 mL H_2O)	$[\alpha]^{20}_D$ (H_2O, 1%)
α	6	973	5.7	13.7	7	14.5	150.5
β	7	1135	7.8	15.3	7	1.85	162.5
γ	8	1297	9.5	16.9	7	23.2	117.4

Source: Shahidi and Han [499].

less than that of the cyclodextrin, it is greater than that of the guest molecule.

The solubility of cyclodextrins can be improved by substituting various hydroxyl groups of the rims of the cyclodextrin molecule [70]. By chemical modification, cyclodextrins may attain some characteristics very different from those of the original material. Cyclodextrins can be incorporated into polymer structures. One such polymer can be produced by linking cyclodextrin rings with suitable agents such as epichlorohydrin in order to obtain insoluble copolymers in the form of water-swelling beads. Some of these polymers retain the ability of the cyclodextrin to form complexes with various compounds, especially those with hydrophobic groups.

It has been reported that if cyclodextrins are linked to polyethers, water-soluble polymers are produced [71]. Initial studies have shown that heptamino-β-cyclodextrin can be cross-linked by hexamethylene diisocyanate. If the degree of polymerization is high enough, the cyclodextrins bound within the matrix become insoluble. The chemical structure of an experimental polymer produced from maize is shown in Figure 34.6.

Polydextrose also designated as poly-D-glucose, a synthetic, highly branched polymer with many glycosidic linkages is produced by heating dextrose with acid and purifying the resulting water-soluble polymer. For instance, melt-condensation of 89% (D-glycose), and 1% citric acid 10%, sorbitol (D-glucitol) results in branched polydextrose with primarily 1→6 linkages and a molar mass of about 22,000 g/mol that is water-soluble [72]. Polydextrose is used as a bulking agent because it is similar to fiber in terms of its resistance to digestion and being tasteless.

34.2.1.5 Sucrose

The most commonly used ingredient in the food industry is sucrose (β-D-fructofuranosyl-α-D-glucopyranoside), which

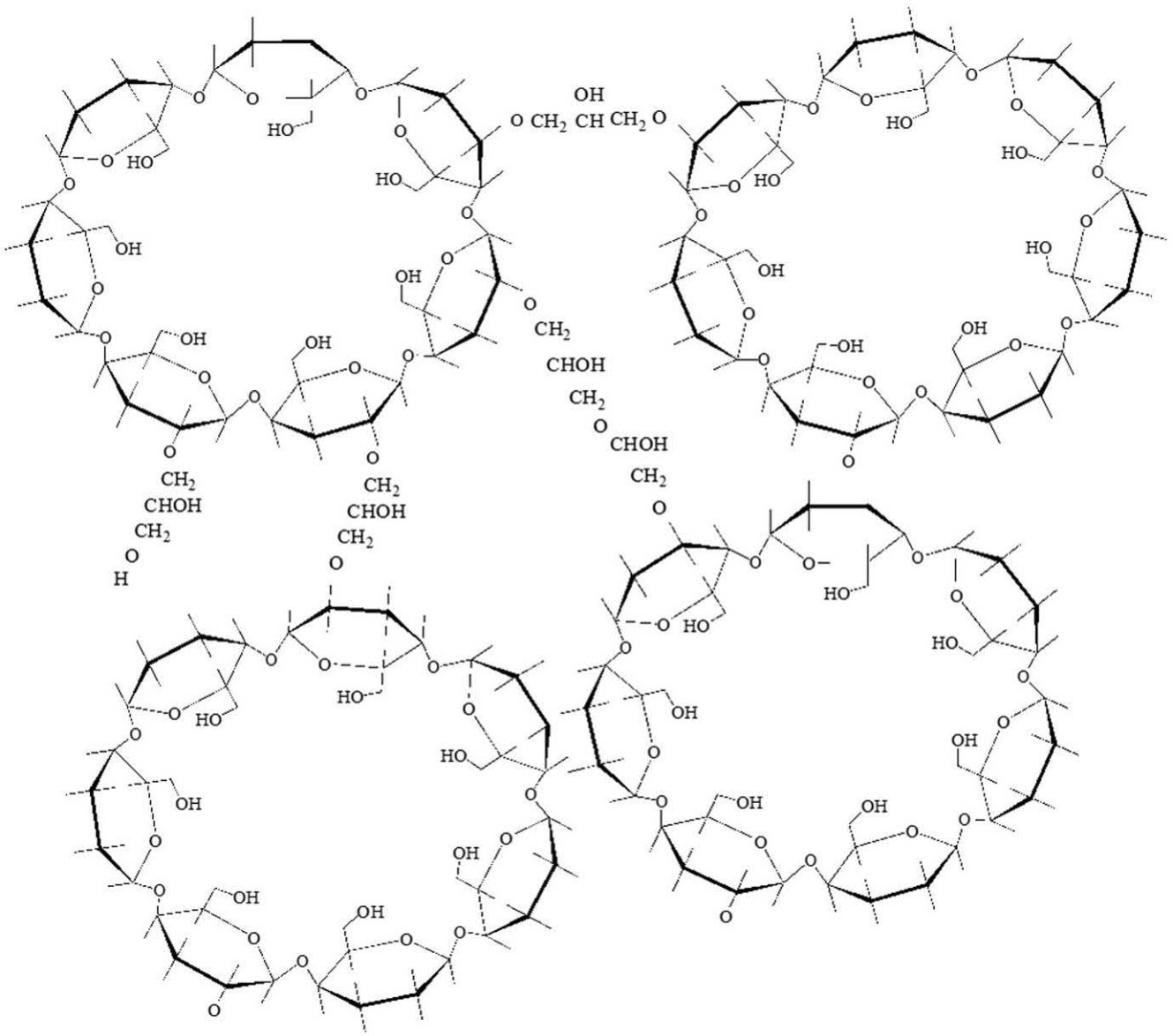

FIGURE 34.6 Structure of a polymeric, modified β-cyclodextrin. (From Pszzcola [497].)

provides sweetness, and acts as a bulking agent, texture modifier, preserving agent, and fermentation substrate in food applications [73]. Sucrose is also useful as a carrier for food ingredient encapsulation because of its (i) quick dissolution in water producing a clear solution, (ii) heat stability, (iii) nonhygroscopicity, (iv) indefinite shelf life under ambient conditions, and (v) inexpensive nature [74].

In extrusion processing, sucrose and other mono- and disaccharides provide flavor, sweetness, energy, texture, stabilization, and control of water activity as well as color [75]. Mixtures of sucrose and maltodextrin are the most commonly used coatings for extrusion encapsulation [76, 77]. Flink and Karel [45] reported that retention of volatiles by carbohydrates during lyophilization was roughly in the order of sucrose > maltose ≥ lactose > glucose >> dextran T-10. In the case of lactose, its crystal form as well as the structure of the volatiles were found to influence the amount of absorption [78]. Sucrose is used for encapsulating food flavors by a process known as cocrystallization [74, 79–81]. However, before it can be used, its chemical structure needs to be modified from a single perfect crystal to that of a microsized, irregular, agglomerated form, prior to cocrystallization occurrence. This modified structure has an increased void space and surface area that provides a porous bed or base for the incorporation of active ingredients.

34.2.1.6 Chitin and Chitosan

Chitin is a β-1,4–linked linear polymer of 2-acetamido-2-deoxy-D-glucopyranosyl residues, and is a main constituent of the exoskeleton of crustaceans such as crab, shrimp, lobster, and crayfish. Chitosan is the principal product from the alkaline hydrolysis of chitin and consists of 2-deoxy-2-aminoglucopyranosyl residues joined by β-(1⟶ 4) linkages. Complex coacervate capsule formation can occur between chitosan, a cationic polyglucosamine, and carrageenan or alginic acid, which are anionic in nature.

Gel bead formation can be achieved by interaction of chitosans with low molecular weight negatively charged counterions such as polyphosphates. The gelling properties of chitosans allow their use in a wide range of applications, the most attractive being coating of foods and pharmaceuticals, and gel entrapment of biochemicals, plant embryos, and whole cells, microorganisms, or algae [82, 83]. Such entrapment offers diverse uses including microencapsulation and controlled release of flavors, nutrients, or drugs. Because chitosan has been shown to be an effective agent, concurrent cell permeabilization and immobilization using chitosan-containing complex coacervate capsules have been explored [82, 83].

Polycationic chitosan molecules can be incorporated with oppositely charged polymers to form coacervate capsules of good mechanical strength. The permeability of these coacervate capsules can be controlled by either altering the type of chitosan and/or the counterion [84].

34.2.1.7 Cellulose and Cellulose Derivatives

Cellulose is the main constituent of plant cell walls and consists of β-(1⟶ 4)-linked glucopyranosyl residues. It is a solid polymer with extended rodlike conformation. In contrast to starch, cellulose is more crystalline. While starch undergoes a crystalline to amorphous transition between 60°C and 70°C in water, cellulose requires about 320°C to become amorphous in water [43]. The primary cell wall of all green plants is made of cellulose and some of its important natural sources include hemp, flax, straw, and jute, among others. In addition, acetic acid bacteria and some forms of algae are known to synthesize cellulose [43].

Together with some other inert polysaccharides (e.g., lignin), cellulose constitutes the indigestible carbohydrate fraction of plant foods, referred to as dietary fiber. Until recently the importance of dietary fibers in human nutrition was considered to be mostly the maintenance of intestinal mobility (peristalsis). However, "functional fiber" refers to isolated, nondigestible carbohydrates that have beneficial physiological effects in humans. Functional fiber may bind bile acids or cholesterol in the intestine, thus preventing their reabsorption into the body. The liver responds by taking up more low-density lipoprotein (LDL) cholesterol from the bloodstream, thereby lowering the concentration of LDL cholesterol in the blood. Short-chain fatty acids, products of fermentation from functional fiber in the gut, may inhibit synthesis of cholesterol by the liver, reducing the concentration of blood cholesterol. The high viscosity of soluble fiber may slow the rate of digestion and absorption of carbohydrates, affecting insulin activity, which is implicated in the removal of LDL cholesterol from the blood.

Cellulose as an edible film for food preservation and other functional ingredients in food processing has attracted much research interest [85–87]. Native cellulose is insoluble in water due to the high level of intramolecular hydrogen bonding in the cellulose polymer [49]. As an edible film for food coatings, the permeability of cellulose coatings can be modified by combining them with other coating materials via etherification [88]; that is, reacting cellulose with aqueous caustic and then with methyl chloride, propylene oxide, or sodium monochloroacetate to yield methylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, and sodium carboxymethylcellulose [49]. Methyl- and hydroxypropyl methylcellulose mixed with lauric, palmitic, stearic, and arachidic acids were found to significantly lower the permeation rate relative to cellulose ether films containing no fatty acids [89]. Cellulose may also be used in the encapsulation of water-soluble food ingredients such as sweeteners and acids as well as for encapsulating enzymes and cells [90].

Methylcellulose (MC) is a hydrophilic white powder that dissolves in water to form a viscous solution. Higher degrees of substitution (DS) and degrees of polymerization (DP) result in lower solubility (Figure 34.7a). Viscosity of MC solutions is stable over pH range of 3–11. Three-dimensional gel formation appears upon heating above 50°C. Methylcellulose has good film-forming properties, but it is not digestible. Synthesis steps contain heating of raw cellulose with sodium hydroxide and treatment with methyl chloride [43].

Hydroxypropyl methylcellulose (HPMC) is similar to methylcellulose and is a white powder or granule that dissolves in water and forms a viscous, nonionic colloidal solution.

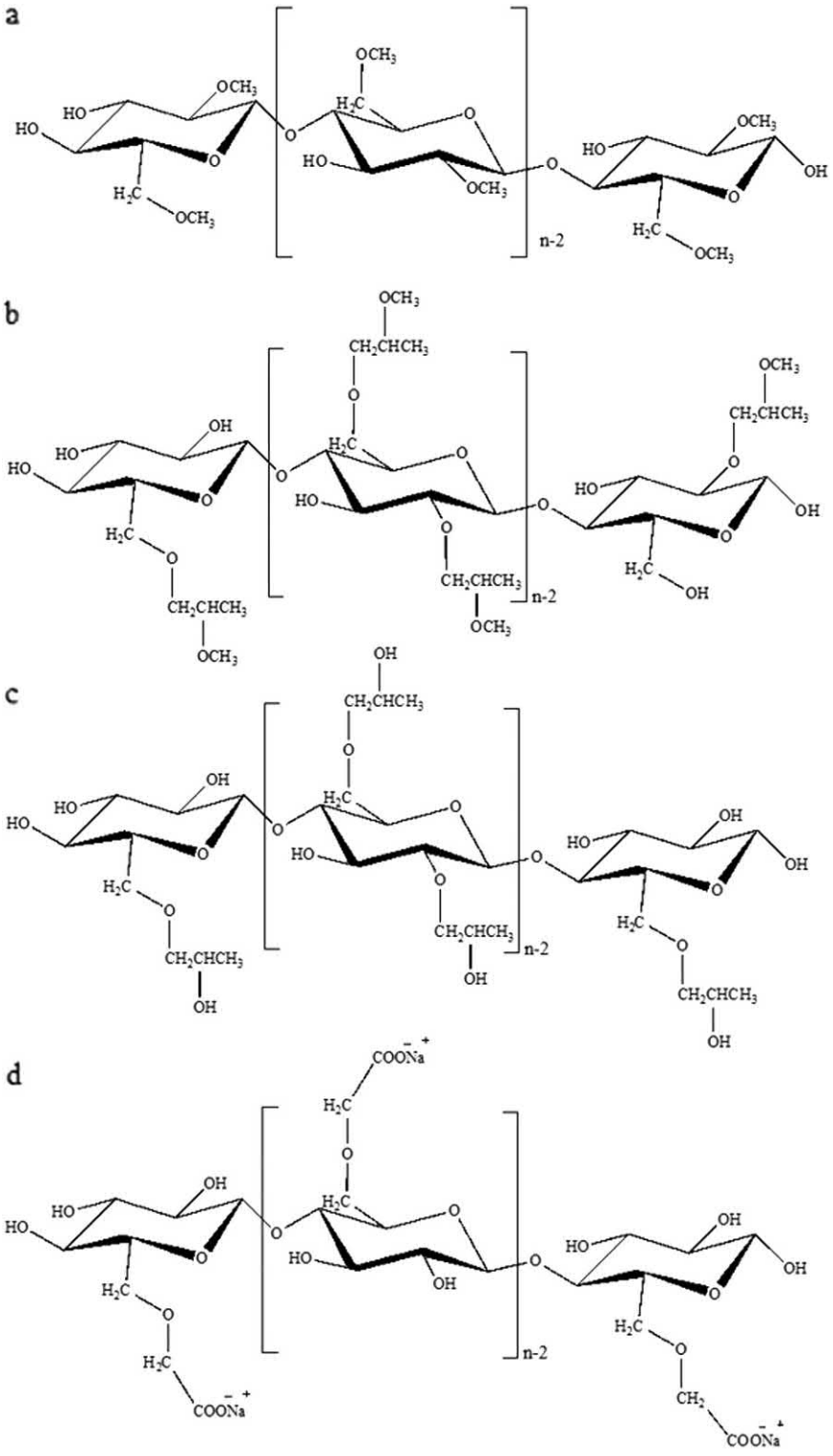

FIGURE 34.7 Chemical structures of (a) methylcellulose (MC), (b) hydroxypropyl methyl cellulose (HPMC), (c) hydroxypropyl cellulose (HPC), and (d) ethyl methylcellulose (EMC). (From Wandrey et al. [43].)

In addition, HPMC is soluble in most polar solvents. They undergo transformation from solution to gel upon heating and cooling. The gel transition temperature usually depends on the ratio of methyl to hydroxypropyl derivatization. The molar mass of HPMC is higher than 10,000 g/mol, with a density of 1.6 g/ml (Figure 34.7b) [91].

Hydroxypropyl cellulose (HPC; Figure 34.7c) is soluble in cold water. Viscosity of the solution could be adjusted by the degree of polymerization. Because of the presence of hydrophilic and hydrophobic groups, it becomes insoluble at temperatures above 45°C and no gel is formed [92, 93]. Advantageous features of HPC include its solubility in ethanol

and mixtures of ethanol and water, and good film-forming ability [43]. The films are glossy, flexible, and nontacky [92].

Ethyl methylcellulose (EMC) behaves like HPMC and MC. EMC is soluble in cold water and forms gels upon heating. Meanwhile, ethylcellulose (EC) is a water-insoluble commercial thermoplast used for coating [43]. Sodium carboxymethylcellulose (CMC) is produced by the reaction of chloroacetic acid and cellulose with alkali. The degree of polymerization decreases a bit during the modification. Modification produces areas of low and high substitution. The product is white to off-white, odorless, and tasteless [94]. CMC dissolves very rapidly in cold water and affords clear and colorless solutions with its viscosity decreasing after heating. The solution behavior of CMC strongly depends on the degree of polymerization and the molar mass. At low pH, CMC might form cross-links between free hydroxyl groups and carboxylic acid (Figure 34.7d) [95].

34.2.2 Plant Exudates and Extracts

Polysaccharide exudates of plants and their extracts are complex macromolecules that are mostly mixtures consisting of oligomers and polymers of different chemical structures. Some of the materials used for food encapsulation are products of gummosis, which are also known as plant gums. Gummosis is a result of stress conditions like heat, wound, and drought. The gums are produced in response to injury of the plant and form the barrier at the lesion hindering the invasion by the microorganisms. Some plant species are cultivated at present to provide the gums for the food industry. Some examples of common plant gums that are harvested include the following: *Acacia senegal* – gum arabic (gum Senegal), *Cyamopsis tetragonolobos* – guar gum, *Astragalus* spp. – gum tragacanth, and *Ceratonia siliqua* – locust bean gum.

Several other tree exudates such as mesquite gum are harvested and consumed [96]. One class of material often exploited for its encapsulating capabilities is that of hydrocolloids, or more commonly, gums. These compounds are long-chain polymers that dissolve or disperse in water to render a thickening or viscosity-building effect [97]. Gums are generally used as texturing ingredients, but their secondary effects include encapsulation capabilities [98], stabilization of emulsions, suspension of particulates, control of crystallization, and inhibition of syneresis (i.e., the release of water from fabricated foods) [99, 100]. Additionally, a few gums are capable of forming gels.

Food gums are obtained from a variety of sources. Although most gums are obtained from plant materials such as seaweed (e.g., carrageenans and alginates), seeds (e.g., locust bean gum and guar gum), and tree exudates (e.g., gum acacia), others are products of microbial biosynthesis (e.g., xanthan gum and gellan gum), and still others are produced by chemical modification of natural polysaccharides. Some commonly used gums as coating materials for food ingredient encapsulation are discussed next.

34.2.2.1 Seaweed Extracts

Alginates, agar, and carrageenan are extracts from red (*Rhodophyceae*) and brown (*Phaeophyceae*) algae, which are collectively referred to as seaweeds [101]. Their use in encapsulation processes is well documented. A major source of industrially produced alginates is the giant kelp, *Macrocystis pyrifera*, which is harvested mechanically off the coast of California. Other seaweed species of alginates include *Laminaria hyberborea*, *Laminaria digitata*, and *Ascophyllum nodosum*. Algae are extracted with alkali from seaweed, and the polysaccharide is usually precipitated from the extract by addition of an acid or calcium salts.

34.2.2.1.1 Alginates

Alginates are salts of alginic acid and include a variety of products made up of poly-β-D-mannopyranosyluronic and poly-α-L-gulopyranosyluronic acids joined in a linear fashion by α -(1⟶ 4) linkages [102]. They are arranged either in regions composed solely of one unit or the other, referred to as M-blocks and G-blocks, or in regions where the two units alternate [101]. In alginates, both the ratio of mannuronic acid to guluronic acid and the structure of the polymer govern how effectively the chains associate during gel formation. Figure 34.8 shows part of the sodium alginate chain. The weight average molar mass of the commercial sodium alginates usually varies between 4×10^4 and 5×10^5 g/mol. However, some alginates have a molar mass higher than 10^6 g/mol. Alginate is available as a dry powder as well as a solution. Degradation of alginate leads to a fast decrease in chain length, which is detectable by a decrease in viscosity. A number of microorganisms are able to digest alginate. Sodium alginate can be kept for years in a deep freezer without any significant degradation. Alginates are found abundantly in nature and are commercially produced mainly from marine brown algae but may also be synthesized as an exocellular material by several bacteria [103].

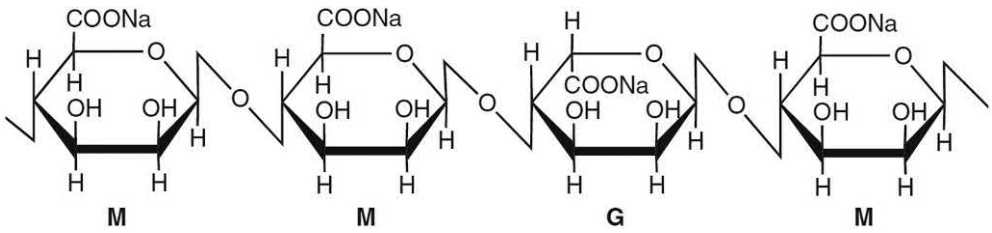

FIGURE 34.8 Chemical structural units of a sodium alginate polymer chain. (From Wandrey et al. [43].)

Alginates are powerful thickening, stabilizing, and gel-forming agents and are utilized in a variety of foods. At a level of 0.25–0.5%, they improve and stabilize the consistency of fillings for baked products, salad dressings, and milk chocolate, and prevent the formation of large ice crystals in ice cream during storage. They are also used as an encapsulating agent. Water-soluble alginate is capable of forming liquid capsules [104]. Alginate films tend to be quite brittle when dry but may be plasticized by inclusion of glycerol [105]. Viscous high-fat food can also be encapsulated with calcium alginate [106].

34.2.2.1.2 Agar

Agar is a heterogeneous complex mixture of related polysaccharides having the same backbone chain structure. Its main components are β-D-galactopyranosyl linked (1⟶4) to a 3,6-anhydro-α-L-galactopyranosyl unit, and partially esterified with sulfuric acid. Deemed as one of the most potent gel-forming agents, agar produces perceptible gelation at concentrations as low as 0.04%. The gelling properties of the gum, the heat resistance of its gels, and the differential between the gel-forming and melting temperatures are the primary reasons for selecting agar. Agar is best known as a culture medium and is not employed to any great extent in foods. Nevertheless, chlorella agar has been used for the encapsulation of flavors [107].

34.2.2.1.3 Carrageenan

Carrageenan is extracted mainly from the red seaweed species, *Chondrus crispus*, with water and a small amount of alkali, followed by filtration and recovery by alcohol precipitation [49]. It is composed of β-D-galactose and 3,6-anhydro-α-D-galactose, which is partially sulfated as 2-, 4-, and 6-sulphates and 2,6-disulphates. The galactose residues are alternatively linked by 1–3 and 1–4 linkages. There are three principal carrageenan fractions known as iota (ι), kappa (κ), and lambda (λ) (Figure 34.9). κ-Carrageenan fraction contains the lowest number of sulfate groups and the highest concentrations of the 3,6-anhydro-α-D-galactopyranosyl units. ι-Carrageenan differs from κ-carrageenan with an additional sulfate group at the 2 position. Meanwhile, λ-carrageenan differs from κ- and ι-carrageenans by having variable amounts of sulfate groups and no 3,6-anhydro-α-D-galactopyranosyl residues [49].

Carrageenan utilization in food processing is based on the ability of the polymer to gel, to increase solution viscosity, and to stabilize emulsions and various dispersions. Gels from carrageenan are thermoreversible. Because of its reactivity with certain proteins, the gum has found use at low concentrations (typically 0.01% to 0.03%) in a number of food products. Locust bean gum is compatible with carrageenan, forming a cooperative association with the double helix structure, and increasing the elasticity of the resultant gels. A process for producing capsules containing meat soup or juice with agar-agar, carrageenan, or pectin coatings has been developed by Hoashi [108].

Carrageenans have typical weight average molar mass in the range of 3×10^5 to 6×10^5 g/mol and are highly polydisperse with a typical number average molar mass of 1×10^5 to 2×10^5 g/mol [109]. Because of the variation in their chemical structures, carrageenans show a wide spectrum of rheological behavior [110]. Depending on the type of carrageenan used, they could form viscous solutions and thermally reversible gels with a texture varying from soft and elastic to brittle. Both ι- and κ-carrageenans have the ability to form elastic gels with some of the cations like K^+ and Ca^{2+} and might be used as adjuncts in encapsulation processes (Table 34.3).

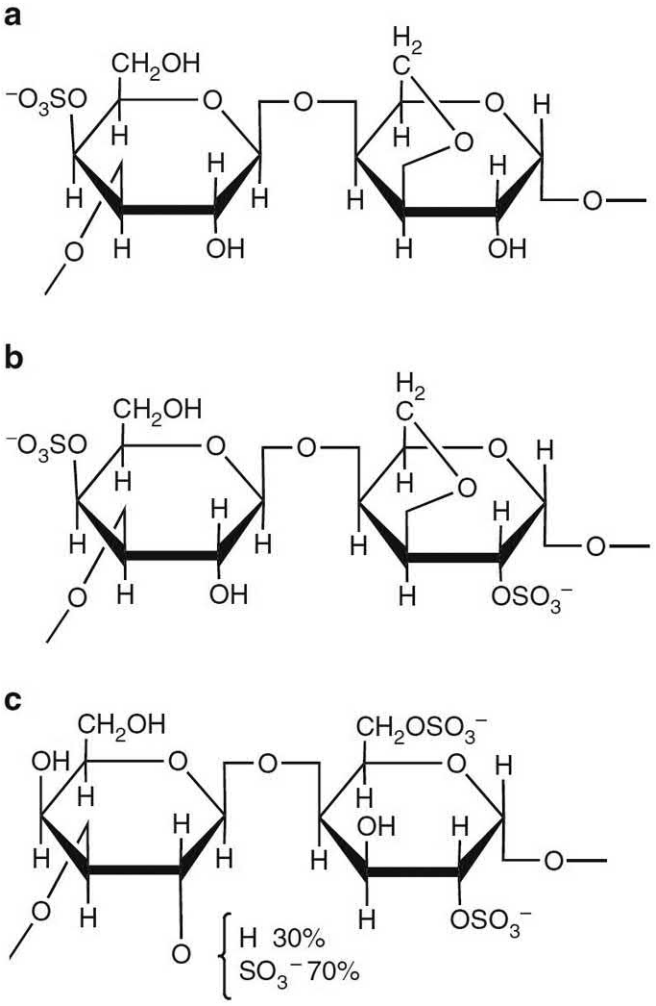

FIGURE 34.9 Repeating dimer units of limit carrageenans: (a) κ-carrageenan, (b) ι-carrageenan; and (c) λ-carrageenan. (From Wandrey et al. [43].)

34.2.2.2 Exudate Gums

Gum arabic (acacia), gum ghatti, gum karaya, and gum tragacanth are referred to as exudate gums. Exudate gums are structurally complex heteropolysaccharides obtained from certain shrubs and small trees that grow predominantly in Africa and Asia [111]. The term *exudate* refers to the fact that gums are secreted or exuded in response to plant tissue injury. Upon exposure to the atmosphere, they form extremely hard, glossy nodules or flakes, which can be harvested [49].

TABLE 34.3
Molecular Structural Properties of Carrageenans

Property	Carrageenan Type		
	Lambda	**Kappa**	**Iota**
Solubility in cold water	All salts soluble; viscous solutions	Na-salts soluble; from limited to high swelling of K^+, Ca^{2+}	Na-salts soluble, Ca-salts give thixotropic dispersions
Solubility in hot water	Soluble	T >70°C	T >70°C
Gelation		Strongest with K^+	Strongest with Ca^{2+}
pH stability	Hydrolysis below pH 4.3	Stable at neutral alkaline pH	pH = 3.5
Salt tolerance	Good	Poor	Good

Source: Davidson (1980).

34.2.2.2.1 Gum Arabic

Gum arabic (GA), which is a natural vegetable colloid obtained by exudation from the trunk and branches of leguminous plants of the Acacia family, primarily *Acacia Senegal*, is the most commonly used encapsulation coating material [112, 113]. Although there are several hundred species of Acacia, only a few are gum producers, and these are located in the subdesert region of Africa. GA is a complex mixture of arabinogalactan oligosaccharides, glycoproteins, and polysaccharides. Its chemical composition could vary depending on the source, climate, age of the tree, season rainfall, time of exudation, and other factors. GA consists of β-(1→3)–linked D-galactopyranosyl units. Side chains are composed of 2-5 β-(1→3)–linked D-galactopyranosyl units and joined to the main chain by 1, 6-linkages. Both the main and side chain contain units of α-larabinofuranosyl, β-D-glucuronopyranosyl, α-L-rhamnopyranosyl, and 4-*O*-methyl β-D-glucuronopyranosyl [114]. GA has a complicated molar mass distribution and some studies have shown that it includes a mixture of higher molar mass hydroxyproline-rich glycoprotein ($M_{w:}$ 2.5×10^6 for the minor component) and lower molar mass polysaccharide (M_w: 2.5×10^5 for the major component) [115, 116].

GA is tasteless, colorless, and odorless, and thus does not affect the color, odor, and taste of the system to which it is added. GA is soluble in both hot and cold water with concentrations of up to 50% (w/w). Viscosity of its solutions varies with the GA type, ionic strength, and pH. Its maximum viscosity is around pH 6 to 7. GA acts as an excellent emulsifier [43] and has the ability to create a strong protective film around oil droplets [117].

34.2.2.2.2 Gum Acacia

Gum acacia is a neutral or slightly acidic salt of a complex polysaccharide with an average molecular weight range of 260–1160 kDa. Gum acacia primarily consists of D-galactopyranosyl, L-rhamnnopyranosyl, L-arabinopyranosyl, L-arabinofuranosyl and D-glucopyranosyluronic acid. It contains ca. 2–3% protein with an arabinogalactan-protein complex and is rich in hydroxyproline, serine, and proline. This protein fraction is responsible for the emulsification properties of the gum. The gum also exists as a mixed salt of sodium, calcium, magnesium, and potassium ions. Owing to the complex character of this polymer, the stereochemical organization of the molecule is not completely understood even though the qualitative and quantitative analysis of the sugars is.

Gum acacia is the traditional gum of choice for flavor encapsulation via spray drying. It dissolves readily in hot or cold water, is an outstanding natural emulsifier, is the least viscous and the most soluble of the hydrocolloids, is stable in acidic media, and rates well based on criteria used in evaluating a flavor carrier. Because beverage applications account for a large proportion of dry flavorings used, emulsion stability in the finished product is one of the most important criteria in carrier selection. Gum acacia has the advantage of being considered natural in virtually all countries. An interesting and unique property of gum acacia is its low viscosity in aqueous solutions. Although solutions containing up to 50% gum can be prepared, the solution viscosity starts to rise steeply at concentrations of greater than 35%. Most other gums yield solutions with high viscosity at concentrations as low as 1%. It would be impossible to effectively atomize these very viscous emulsions, and thus these other gums are not useful, especially as flavor encapsulants.

Gum acacia is also applied as a flavor fixative in the production of powdered aroma concentrates. Although modified food starches are superior to traditional gum acacia in emulsion stability, gum acacia produces quite stable emulsions. The emulsions are then spray dried. In this process, the polysaccharide forms a film surrounding the oil droplet, which then protects the oil against oxidative degradation. Compared to maltodextrins, gum acacia gives superior aroma retention during drying and very little aroma is lost during storage at humidities below the water monolayer level [118]. A new generation gums (blends of West Africa gums) are superior even to modified starches for stabilizing flavor emulsions [48]. Protection of oxidizable flavorings by gum acacia varies with the source of the gum. The traditional gum acacia is not quite as good as the modified food starch/corn syrup solids blend

and quite inferior to the blends of West African gums [48]. Blends of gum acacia with maltodextrins and the new West African gum acacia can be used to encapsulate flavors and offer excellent stability to oxidation [119].

34.2.2.2.3 Gum Ghatti

Gum ghatti is an amorphous translucent exudate of the *Anogeissus latifolia* tree of the family Combretaceae, which is native to India [105]. It is a water-soluble, complex polysaccharide consisting of L-arabinofuranosyl, D-galactopyranosyl, D-mannopyranosyl, D-xylopyranosyl, and D-glucopyranosyluronic acid units. As a whole, it is nongelling but can be dispersed in hot or cold water to give a colloidal sol that develops due to a soluble fraction. The gum closely resembles the viscosity and emulsifying properties of gum acacia and has been effectively used in food systems as an emulsifier and a stabilizer. Gum ghatti is seldom used because of its limited supply [101].

34.2.2.2.4 Gum Karaya

Gum karaya is the dried exudate of the *Sterculia* tree, which grows in central and northern India, hence it is routinely referred to as Indian gum. It occurs naturally as a complex, partially acetylated branched polysaccharide containing about 37% uronic acid residues and approximately 8% acetyl groups. Gum karaya comprises a central chain of D-galactopyranosyl, L-rhamnopyranosyl, and D-galactopyranosyluronic acid units, with some side chains containing D-glucopyranosyluronic acid [105]. It swells very easily in water and absorbs water very rapidly to form viscous colloidal dispersions at low concentrations. The gum forms smooth films that can be plasticized with compounds such as glycols to reduce brittleness [49].

Gum karaya has a molar mass of up to 1.6×10^7 g/mol, and a branched structure [120]. It consists of α-L-rhamnose residues and α-D-galacturonic acid. The side chains are attached by (1→2)- or (1→3)-linkages of β-D-galactose and/or β-D-guluronic acid to the galacturonic acid of the main chain. Moreover, half of the rhamnose residues of the chain are (1→4)-linked to β-D-galactose units [121]. Powdered gum karaya is white to grayish-white, and it is one of the least soluble of the gums. About 10% of the native gum is soluble in cold water and this percentage increases to about 30% in hot water. However, after deacetylation, 90% of it dissolves in water [114]. India is the largest producer of gum Karaya, so it is sometimes known as Sterculia gum, which is a dried exudate from the branches and stems of *Sterculia urens Roxbourg* and other species of *Stericula* [122].

34.2.2.2.5 Gum Tragacanth

Gum tragacanth is the dried gum exuded from the stems of *Astragalus gummifer* and other Asiatic species of *Astragulus*. The plants grow wild in certain areas of Asia Minor and in the arid and mountainous regions of Iran, Syria, and Turkey. Gum tragacanth has a complex structure and upon hydrolysis yields D-galacturonic acid, L-fucose, D-galactose, D-xylose, and L-arabinose. According to Glicksman [105], it consists of a mixture of a water-insoluble component, tragacanthic acid that constitutes 60–70% of the gum and a water-soluble component, tragacanthin or arabinogalactan, in which L-arabinose is the predominant sugar. The water-soluble to water-swellable ratio varies, being, for example, 35:65 and 60:40. The tragacanthic acid fraction has a rodlike molecular shape and a very high molar mass [123]. The major chain is formed by (1→4)-linked D-galactose residues and side chains of D-xylose units attached to the main chain via (1→3)-linkages. Water-soluble tragacanthin is a neutral, very highly branched arabinogalactan with a spherical molecular shape. Its structure might consist of a core composed of (1→6)- and (1→3)-linked D-galactose and attached chains of (1→2)-, (1→3)-, and (1→5)-linked L-arabinose [114, 122].

Gum tragacanth is an acid-resistant gum and does not degrade at low pH. It is also considered as a bifunctional emulsifier for two reasons: its ability to lower the interfacial tension between the oil-in-water emulsion and to increase the viscosity of the aqueous phase. Gum tragacanth forms viscous aqueous solutions even though at low concentrations [124]. It swells rapidly in either cold or hot water to form highly viscous colloidal sols or semigels, which act as protective colloids and stabilizing agents. The gum is generally used as a thickener and stabilizer in salad dressings, sauces, bakery emulsions, toppings, ice cream, and confectioneries.

34.2.2.2.6 Mesquite Gum

Mesquite gum is a neutral salt of a complex acidic branched polysaccharide. It is formed by (1→3)-linked β-D-galactose residues with (1→6)-linked branches, L-rhamnose, β-D-glucuronate, and 4-*O*-methyl β-D-glucuronate. It also contains a small amount of a protein (0.7–5.8%) [125–129]. Solubility of Mesquite gum is similar to GA and has a dark brown color compared to GA. Its molar mass is 3.5×10^4 to 9.3×10^5 g/mol with a protein content of about 0.04% to 30%. Solutions of up to 50% concentration may be prepared, however, an increase of viscosity with increasing concentration is very high for mesquite gum compared to GA [130]. Mesquite gum has perfect film-forming properties [131] and is obtained from the mesquite tree *Prosopis* spp. or shrub that grows in the southwest of Mexico, the United States, and other areas in the world [96].

34.2.2.2.7 Galactomannans or Locust Bean Gum

Galactomannan or locust bean gum (LBG), also called guar gum or carob bean gum, are galactomannans [132]. They consist of linearly (1→4)-linked β-D-mannopyranosyl units and with single α-D-galactopyranosyl units connected by (1→6) linkages. Three gum types, namely, tara gum, LBG, and guar gum, vary in the ratio of D-mannosyl to D-galactosyl. In tara gum, every third main chain unit bears a side unit, whereas in LBG it is every fourth, and in guar gum it is every second main chain unit (Figure 34.10). The actual ratios differ slightly and vary from the average for LBG of between about 3.9:1 and 3.5:1 [133].

The three gum types have different in solubilities. Solubility increases with increasing the number of side units. Solubility of tara gum is about 70% under these conditions but

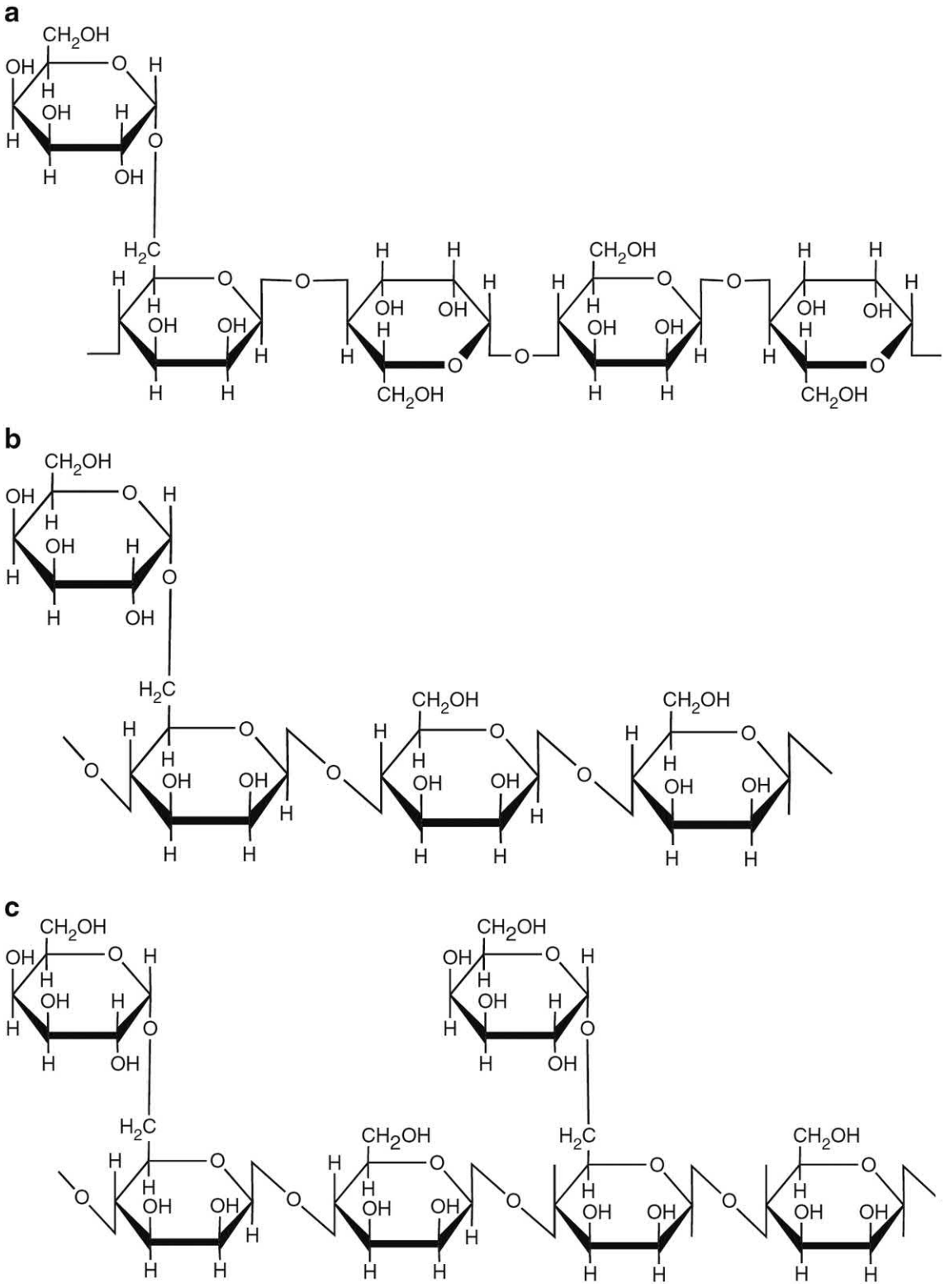

FIGURE 34.10 Chemical structures of (a) locust bean gum, (b) tara gum, and (c) guar gum. (From Wandrey et al. [43].)

is fully soluble above 70°C. However, guar gum is completely water-soluble at room temperature, and LBG has a limited water solubility at ambient temperature and swells below 60°C but becomes soluble above 60°C and fully hydrated if heated for 10 min at 80°C [133]. The range of molar mass for guar gum varies from 1.5×10^5 to 1.5×10^6 g/mol, having a higher molar mass as well as producing higher viscosities of solutions than LBG. Moreover, purification, isolation, solution preparation, and characterization method could have an impact on their molar mass [134–136].

34.2.2.3 Pectins

Pectin is a high molar mass heteropolysaccharide of about 65% (wt) of a-(1→4)-linked D-galacturonic acid-based units. All units are present as free acid (potassium calcium, sodium, ammonium), as acid amide in amidated pectins, or esterified with methanol. In addition, some of the neutral sugars like L-arabinose L-rhamnose, D-galactose, D-xylose, as well as small amounts of others are part of this polymeric chain. L-Rhamnose units exist as (1→2)-linked in the main chain, while other neutral sugar residues are bonded preferably to the rhamnose [72].

Pectins may have different degrees of esterification of the carboxyl groups of galacturonic acid, generally between 20% and 80%. Pectins with low esterification or low methoxylation (LM) have less than 50% ester groups. However, pectins with more than 50% esterification are high-esterified or highly methoxylated (HM). The molar mass depends on the source of pectin as well as the processing and varies from 10^4 to 2×10^5 g/mol [137]. Pectins are insoluble in most organic solvents but soluble in water. Solutions could be obtained up to concentrations varying from 6% to 12%, depending on the type, and have a low viscosity compared to other plant gums [43].

34.2.3 Microbial and Animal Polysaccharides

Polysaccharides that are produced biotechnologically by bacteria are biopolymers with partially unique functional properties such as xanthan and gellan. A brief description of xanthan and gellan polysaccharides is provided in the following sections.

34.2.3.1 Xanthan

Xanthan is a high molar mass anionic polyelectrolyte that occurs as a mixed salt of sodium, calcium, and potassium. The chemical structure of xanthan consists of β-(1→4)-D-glucopyranosyl units with every second unit having a trisaccharide side chain attached at the C-3 position, one D-glucuronosyl unit between two D-mannosyl units (Figure 34.11). Approximately 40% to 50% of the terminal mannosyl units are 4, 6-pyruvated and the nonterminal mannosyl units are 6-acetylated. Their molar mass varies from 1.5×10^5 g/mol to several millions. Association phenomena observed for xanthan gum could explain these differences [138, 139]. Xanthan is soluble in cold water and hydrates quickly in it without lumping. Generally, its solution viscosity is retained at pH 2 to 12 [140].

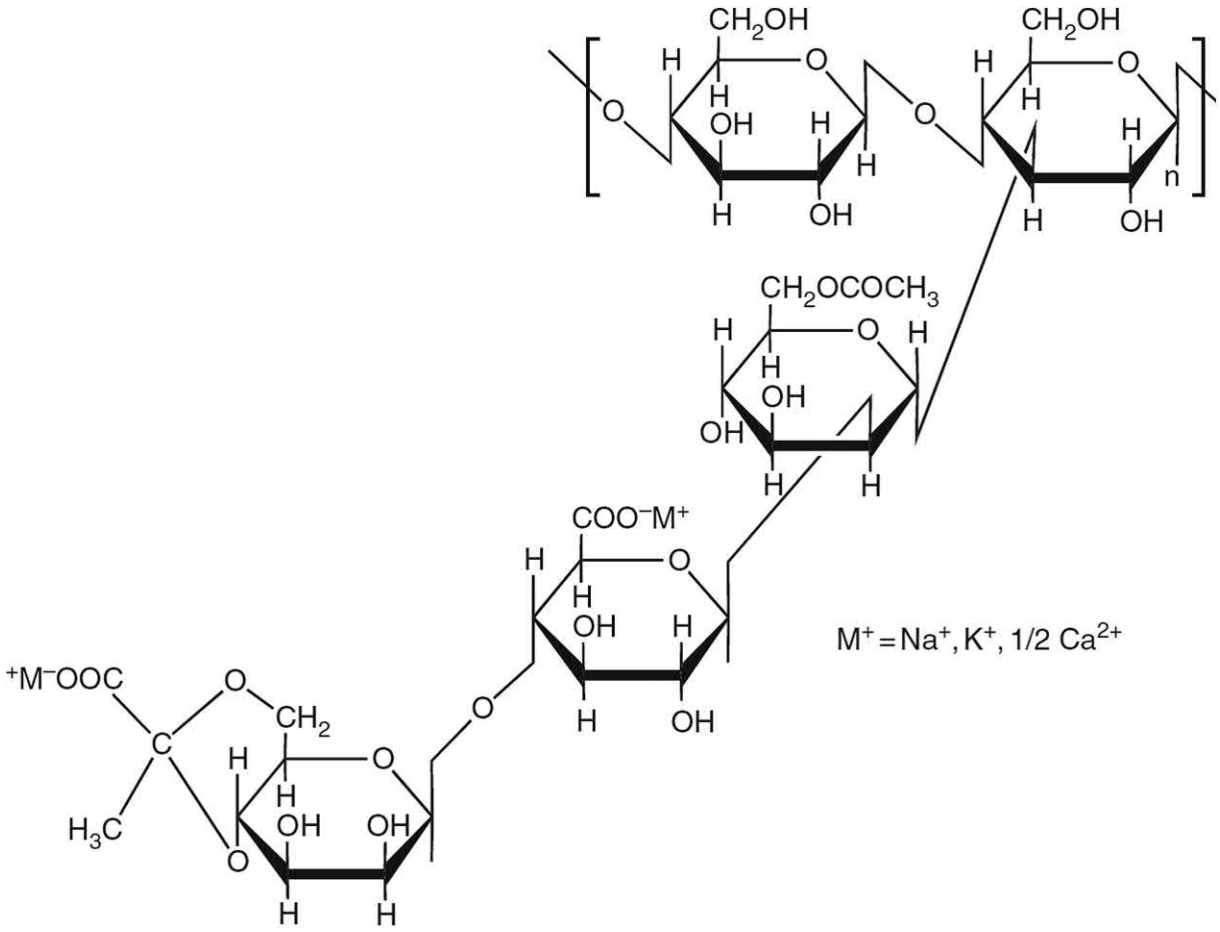

FIGURE 34.11 Chemical structural of xanthan gum. (From García-Ochoa et al. [498].)

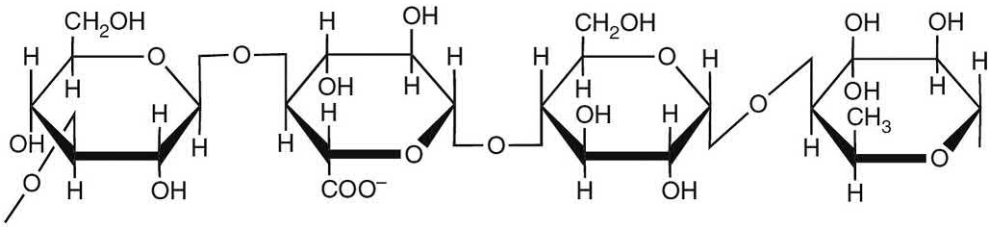

FIGURE 34.12 Chemical structural of gellan gum. (From Wandrey et al. [43].)

34.2.3.2 Dextran

Dextrans are linear polymers of α-D-glucose linked by α-(1→6) glycosidic bonds and can have variable amounts of α-(1→3) branches. The links in the main chain can be α-(1→2) links or α-(1→4) instead of α-(1→6) links. The molar mass of dextrans is in the range of 3×10^3 to 2×10^5 g/mol and they are freely soluble in water. Dextran produced from sucrose by microbial fermentation processes can be easily isolated. The bacteria used in the fermentation process determine the chain architecture and products so prepared are available commercially as a powder or solution [72].

34.2.3.3 Gellan

Gellan consists of one glucuronic acid, one rhamnose, and two glucose units. The 3-linked glucose unit is substituted with glyceryl at O 2 and with acetyl at O 6 (Figure 34.12). Thus, it can be designated as (1→4)-L-rhamnopyranosyl-α-(1→3)-D-glucopyranosyl-β-(1→4)-D-glucurono-pyranosyl-β-(1→4)-D-glucopyranosyl-β-(1→ 4) [43]. Molar mass of gellan is up to 5×10^5 g/mol. Solubility and solution properties of gellans depend on both the degree of substitution and concentration of ions present in the solution [43].

34.2.4 Lipids

Lipids include edible oils, waxes, fats, and phospholipids. These are widely distributed in nature and are water-insoluble. A cursory discussion of this topic is provided in the following sections.

34.2.4.1 Fatty Acids and Fatty Alcohols

There are two types of fatty acids that might be distinguished: saturated and unsaturated. Saturated fatty acids are linear monocarboxylic acids with the general formula of $CH_3(CH_2)_nCOOH$, while unsaturated fatty acids have one or more double bonds in their chain. They differ in the number of carbons in the aliphatic chain and the number of double bonds present [43, 141].

In general, short-chain aliphatic acids are miscible in water; furthermore, the water solubility rapidly decreases with increasing chain length. Melting points of fatty acids differ over a wide range. For example, for the saturated acids, which have much higher melting point, stearic acid (n = 16) is 69.4°C and palmitic acid is (n = 14) 62.6°C. Fatty acids tend to undergo autoxidation at room temperature [142]. Fatty acids are produced by the hydrolysis of the ester linkage of naturally occurring oils and fats. In addition, the glycerol is obtained as the by-product of this process [43].

34.2.4.2 Acylglycerols (Glycerides)

Monoglyceride (monoacylglycerol), diglyceride (diacylglycerol), and triglyceride (triacylglycerol) belong to the family of acylglycerols (Figure 34.13). Only one, two, or three fatty acid chains are covalently bound to a glycerol molecule by ester linkages. The fatty acids may be unsaturated or saturated. Glycerides are generally insoluble in water. Monoglycerides and diglycerides have emulsifying properties. The melting points of the glycerides depend strongly on the symmetry of the fatty acid residues and their chemical nature [143].

Acetylation of glycerol monostearate, by its reaction with acetic anhydride, yields 1-stearodiacetin. This acetylated monoacylglycerol displays the unique characteristic of solidifying from the molten state into a flexible, waxlike solid. It is found that the barrier properties of acetoacylglycerol improve as the degree of acetylation increases. This is due to the removal of free hydroxyl groups, which would otherwise interact directly with migrating water or other small polar molecules. The lower permeability through the acetoacylglycerol film prepared from technical grade monoacylglycerols might be a consequence of difference in crystal packing or the number of free hydroxyl groups [144]. Although the water vapor permeability of acetylated monoacylglycerol films is considerably less than that of most polysaccharide films, it is greater than the permeability values of ethyl- and methylcellulose [145].

34.2.4.3 Waxes: Beeswax, Candelilla Wax, Carnauba Wax

Waxes are important derivatives of higher alcohols, such as C_{12}–C_{28}, which are esterified to long-chain fatty acids.

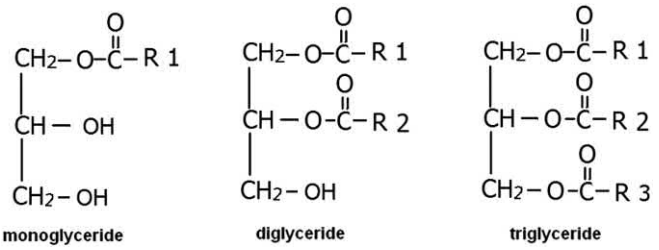

FIGURE 34.13 Acylglycerols (monoacylglycerol or monoglyceride; diacylglycerol or diglyceride; and triacylglycerol or triglyceride), also known as glycerides.

Traditionally, wax coatings have been applied to fresh fruits and vegetables to extend their postharvest storage life. Edible waxes are significantly more resistant to moisture transport than most other lipid or nonlipid coatings. It has been reported that waxes are most effective in blocking moisture migration; paraffin wax being the most resistant followed by beeswax [144, 146, 147]. For this reason, waxes are commonly used as lipid coatings for encapsulation of food ingredients, particularly for the encapsulation of water-soluble ingredients. In 1980, Petroleum wax was permitted for use by the US Food and Drug Administration (USFDA) in formulating microcapsules for encapsulation of spice-flavoring substances in frozen pizza [148].

The great resistance of paraffin and beeswax coatings to diffusion of water is related to their molecular compositions. Paraffin wax consists of a mixture of long-chain saturated hydrocarbons, whereas beeswax comprises 71% hydrophobic, long-chain ester compounds, 15% long-chain hydrocarbons, 8% long-chain fatty acids, and 6% other compounds [149, 150]. The absence of polar groups in paraffin and the relatively low level in beeswax account for their significant resistance to moisture transport. Main components of beeswax are paraffin (about 15%), triacontylcerotinate (approximately 10%), and triacontylpalmitate (about 75%) [142]. In general, waxes are practically insoluble in water. Beeswax melts in the range of 62°C to 64°C, and the color of beeswax varies from nearly white to brownish [43].

Candelilla wax is soluble in several organic solvents and melts in the range of 67–79°C. The color of candelilla wax is light brown to light yellow. It is compatible with all animal and vegetable waxes, fatty acids, and a large variety of natural and synthetic resins. Carnauba wax is one of the hardest natural waxes and has a melting point (about 83°C) higher than that of candelilla wax [43]. Waxes are isolated from plant and animal products. Candelilla wax is derived from leaves of the Candelilla shrub that grows in northern Mexico. Meanwhile, carnauba wax is obtained from leaves of palm trees in Brazil. Beeswax is secreted by young honey bees to construct the honeycomb [43].

34.2.4.4 Lecithins

Lecithin plays a significant role as a surface-active substance in the production of emulsions. Pure lecithin is a water-in-oil (W/O) emulsifier with a hydrophile–lipophile balance (HLB) value of about 3. Because commercially used lecithins are complex mixtures of lipids, their HLB values vary considerably.

Major phospholipids of raw soybean lecithin are listed in Table 34.4 [151]. The ethanol-insoluble fraction is suitable for stabilization of W/O emulsions and the ethanol-soluble fraction for O/W (oil-in-water) emulsions. To increase the HLB value, "hydroxylated lecithins" are prepared by controlled partial oxidation of unsaturated acyl residues with hydrogen peroxide or benzoyl peroxide [151].

Lecithin vesicles have recently been used for encapsulation of food enzymes since the formation of lecithin capsules can be achieved under a relatively low temperature. Using lecithin vesicles to encapsulate lysozyme and pepsin, it was found that the encapsulating efficiency was best when the pH was close to the isoelectric point of each enzyme [152].

TABLE 34.4
Percentage of Phosphatidyl Compounds in Unfractionated and Fractionated Soy Lecithin

Type	Unfractionated	Ethanol-Soluble Fraction	Ethanol-Insoluble Fraction
Phosphatidylethanolamine	32.6	32.5	32.6
Phosphatidylcholine	32.6	65.1	4.6
Phosphatidylinositol	34.8	2.4	62.8

Source: Belitz and Grosch [151].

If blended with other coating materials, lecithin changes the structure of microcapsules formed. Studies by Matsuzki et al. [153] on the encapsulation of β-galactosidase in lecithin-cholesterol liposomes prepared by dehydration–rehydration (DR) and reverse-phase evaporation (RE) revealed that encapsulation efficiency decreased as cholesterol content increased. A mixture of lecithin and polyethylene has been used for encapsulating active ingredients such as sweeteners and flavor compounds [154]. Furthermore, lecithin itself has also been encapsulated as a dietary supplement [155].

34.2.4.5 Liposomes

A liposome (or lipid vesicle) is defined as a structure compound of lipid bilayers that encloses a number of aqueous or liquid compartments [156]. Prepared by a variety of techniques, liposomes consist of one, a few, or many concentric bilayer membranes whose diameter size varies from about 25 nm to several micrometers (Figure 34.14). Figure 34.15 shows the general chemical structure of one of the main phospholipids/phosphodiacylglycerols, namely, phosphatidylcholine. Phospholipids contain two long-chain fatty acids. The third hydroxyl group of the basic glycerol is modified with the phosphoric acid linked to a base such as an ethanolamine or choline [142]. As with triacylglycerols [43], numerous species are possible by variation of the different head groups and fatty acyl substitution at the second and first position of the glycerol backbone.

The most abundant phospholipid is lecithin [157], where the base is choline. The fatty acid components may be unsaturated or saturated, such as stearic, palmitic, oleic, linoleic, or linolenic acid. Other polar lipids are cephalins where the base is ethanolamine (phosphatidylethanolamine). Over the past few decades, liposomes have been studied extensively in the medical and pharmaceutical areas because of their potential use as targetable carriers of drugs and bioactive macromolecules [158]. Liposome microencapsulation technologies have been developed almost to the point where they can be employed in a variety of commercial applications. Recently, there has been interest in use of liposomes in the food industry for development of new food products with improved

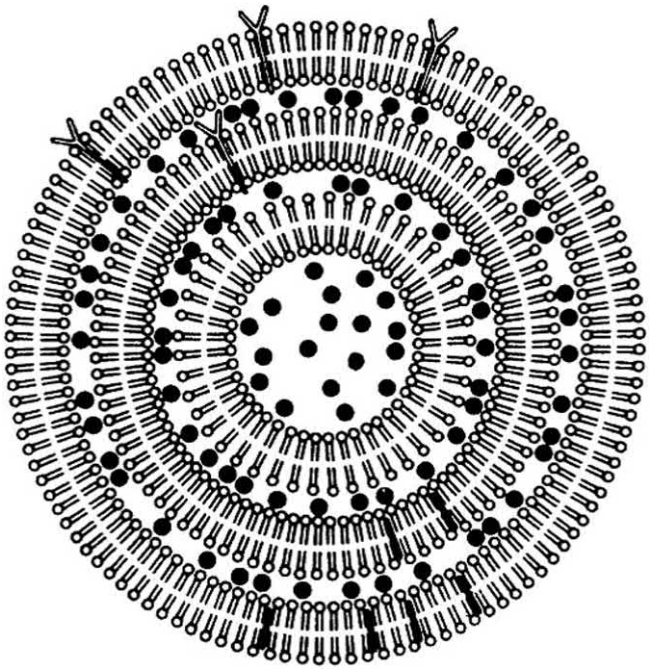

FIGURE 34.14 Schematic structure of a liposome. (Adapted from Shahidi and Han [499].)

characteristics, especially for encapsulation or immobilization of enzymes.

Phospholipids generally form liquid crystalline suspensions as long as the temperature is held at above the phase transition. Because of the amphiphilic character, they function well as emulsifying and dispersing agents. Thus, when mixed with water, they self-assemble or aggregate into well organized and defined bilayers and structures [157]. Liposomes are prepared from phospholipids such as those from egg yolk or soybean lecithin. Semisynthetic phospholipids with fatty acid chains of defined length and saturation as well as cholesterol are also used for specific purposes. The choice of the type of phospholipid and the amount of cholesterol play important roles in determining liposomal stability upon storage and their fate in injected animals [158]. Virtually any substance regardless of solubility, electrical charge, molecular size or other structural characteristics can be incorporated into liposomes, provided that the substance does not interfere with liposome formation [158]. Water-soluble materials may be entrapped in the aqueous phase of liposomes, whereas lipid-soluble materials will be incorporated into the lipid phase. Liposome structure is determined by its method of preparation. Although various techniques exist for preparing liposomes [159, 160], they are generally divided into three classes, namely, multilamellar vesicles (MLV), small unilamellar vesicles (SUV), and large unilamellar vesicles (LUV).

Multilamellar vesicles were first prepared by Bangham et al. [161]. In a typical preparation, a solution of phospholipids in chloroform is evaporated producing a thin film, which is then hydrated with an aqueous solution. The main advantage of MLVs is that the lipid and the aqueous solution to be encapsulated are not subjected to harsh treatments such as exposure to organic solvents or to high-intensity ultrasound. However, a major disadvantage of MLVs is their heterogeneous size distribution (diameters in the range 0.2–2.0 μm) and their low encapsulation efficiency (5–14%) [162].

Small unilamellar vesicles were first prepared from MLVs by sonication. High-intensity ultrasound results in MLVs of a much smaller size (25–50 nm in diameter). A second method for producing SUVs involves injection of lipids, dissolved in ethanol into the desired aqueous phase. The resulting vesicles had diameters in the range of 30–110 nm, while a third technique involves pumping of MLVs through a French pressure cell to produce liposomes with diameters in the range of 30–50 nm [160]. The main disadvantage of SUVs is their small diameter and consequently their low capture volume.

Several methods are available for production of LUVs whose size range from 100–500 nm; these are often the most useful liposomes. The three common methods of preparing LUVs are infusion, reverse-phase evaporation, and detergent dilution. In general, LUVs are more homogeneous than MLVs and have a higher encapsulation efficiency than SUV.

The gel-to-liquid-crystalline transition temperature is one of the key parameters for liposomal systems, where the bilayer loses a considerable amount of its ordered packing structure because of the melting of hydrocarbon chains of the lipids. Liposomal content might leak out fast, especially above the gel-to-liquid-crystalline temperature. Furthermore, liposomal functionality and properties depend on external parameters such as ionic strength and pH of the medium [157]. Phospholipids are present in almost all plant and animal cells. Commercially, they are isolated from several sources such as soybean oil and egg yolk [163]; however, they are also produced from milk fat globules isolated from buttermilk [164].

A serious drawback of the aforementioned liposome preparations for their application in foods has been the use of organic solvents. Liposome microencapsulation using a microfluidizer eliminates this problem because the method does not utilize any organic solvent or detergent. The two most common microencapsulation techniques, spray drying and extrusion, encounter major problems with flavor encapsulation, the

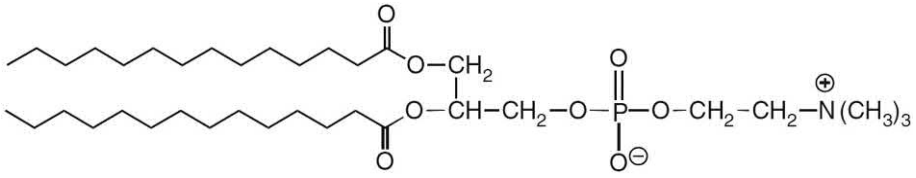

FIGURE 34.15 Phospholipid (e.g., phosphatidylcholine).

occurrence of oxidative reactions, and inability to implement procedures for intermediate-moisture foods [156]. A limitation of the use of liposomes in some food applications may be their lack of stability in the presence of moderate levels of oils or hydrophobic proteins.

34.2.5 Proteins

As an important nutrient in food, proteins possess many desirable functional properties. These properties allow them to be good candidates as coating material for the encapsulation of food ingredients, even though food hydrocolloids are mostly used for this purpose. Gelatine is the most commonly used protein for this purpose, but other proteins such as sodium caseinate, whey protein and soy concentrate, and soy protein isolates have been utilized [165]. Protein-encapsulated tallow and vegetable oils have been applied to produce animal feed [166]. Proteins can also be used, together with other coating materials, to form microcapsules. A mixture of protein and carbohydrate has been applied to an encapsulation process of oily substances [167, 168].

34.2.5.1 Gelatine

Gelatines are water-soluble molecules that vary widely in their size and charge distribution. Nevertheless, they generally have a characteristic primary structure determined by the parent collagen [169]. Gelatine derived from pig skin consists of 33 glycine, 11 alanine, 13 proline, 9 hydroxyproline, 3.5 serine, 5 arginine, 3 aspartic acid, 2.5 glutamic acid, 3 lysine, and also leucine, valine, threonine, phenylalanine, isoleucin, hydroxylysine, histidine, tyrosine, and methionine. However, this can vary depending on the source of raw material as well as the processing technique employed. In type B gelatines, glutamine and aspargine are missing due to their conversion into glutamic acid and aspartic acid. The molar mass of gelatine is less than 5×10^4 g/mol [169]. Gelatines do not occur naturally but are manufactured from collagens by destroying the secondary and higher structures of collagen. Higher structures of collagen are the major constituent of all white fibrous connective tissue occurring in animals such as cartilage, skin, and sinews. The manufacturing process of gelatine is complex and uses skin, bone, or hide from all domesticated cattle, pigs, and horses as preferred sources; fish skin may also be used as a starting material source [43].

Gelatine that is derived from collagen is a valuable coating material partly because it is nontoxic, inexpensive, and commercially available in nature. In addition to good film-forming properties, gelatine has other ideal chemical and physicochemical characteristics that lend themselves to microencapsulation. For example, gelatine forms thermally reversible gels when warm aqueous suspensions of polypeptides are cooled. Fish skin gelatine has a lower gelation temperature of about 5°C for cold-water fish skin gelatine, while mammalian gelatines dissolve in hot water, thus forming solutions of high viscosity [170]. With an aqueous solution of gelatine, the change between the gel and solid state is quite definite. However, when the gelatine concentration in the aqueous solution is lower than about 1%, definite gelation cannot be observed even by cooling. These characteristic properties are effectively used for formation of capsules. The isoelectric point of gelatine and its derivatives can be changed depending upon the method of preparation [171]. By changing the pH of the aqueous solution, either polycationic or polyanionic effects are exhibited by gelatine. This property is used for coacervation formation.

Gelatine is often used in combination with gum acacia to form coating films. Gum acacia, a hydrocolloid derived from plant sources, consists mainly of carboxylic acid functional groups. When the pH is lower than its isoelectric point, gelatine becomes polycationic, and hence, there is an interaction between polycationic gelatine and polyanionic gum acacia, resulting in the formation of a coacervation. As an example, if pigskin gelatine (isoelectric point pH 8–8.5) in aqueous solution is mixed with gum acacia at pH 4.0–4.5, a complex coacervation will form because of the charge attraction between the negatively charged acacia gum and the positively charged gelatine [171]. Fixing (insolubilization) of this structure can be achieved by the use of cross-linking agents such as ionized calcium. The type of gelatine and gum acacia selected, and the formation and fixing procedures employed ultimately influence coating permeability [171]. Coating formation can also be achieved by a solvent evaporation technique.

34.2.5.2 Gluten

Gluten is a complex mixture of gliadins (monomeric gluten) with a molar mass of about 3×10^4 to 8×10^4 g/mol [172] and glutenins (polymeric gluten) with a molar mass of approximately 8×10^4 to several million g/mol [173]. Gliadin is a highly heterogeneous mixture of monomeric gluten. There are three groups of gliadins: α-, γ-, and ω-type. The ω-type gliadins are classified as sulfur-poor prolamins, whereas the α- and γ-types are classified as sulfur-rich prolamins [174].

Wheat glutenin is a heterogeneous mixture of disulfide-stabilized polymers of low-molar-mass glutenin subunits (LMM-GS) and high-molar-mass glutenin subunits (HMM-GS). Recently, more than 19 different HMM-GS have been identified [175]. Glutenin has low solubility in water because of its higher content of nonpolar amino acids and glutamine, lower content of amino acids with ionizable groups, and their high molar mass [176]. Both glutenin and gliadin exist conjoined with starch, in the endosperm of several grass-related grains such as wheat, barley, and rye [177].

34.2.5.3 Milk Protein

Bovine milk consists of water, lactose, fat, other minor components, and nearly 3.0% to 3.6% proteins (caseins and whey proteins are the two major fractions) [178–181]. These can be used in different ways in a variety of food formulations.

34.2.5.3.1 Caseins

The most predominant phosphoprotein found in milk is casein. Four principal primary proteins of the highly heterogeneous casein fractions are αs_1-, αs_2-, β-, and κ-casein (Table 34.5). Caseins may differ in their net charge, metal binding,

TABLE 34.5
Typical Characteristics of Casein Fractions of Bovine Milk

Parameter	Caseins			
	α_{s1}-Casein	α_{s2}-Casein	β-Casein	κ-Casein
Concentration (wt%)	0.9–1.5	0.3–0.4	0.9–1.1	0.3–0.4
Molar mass ($\times 10^{-4}$ g/mol)	2.36	2.52–2.54	2.4	1.9
Isoionic pH	4.94	5.45–5.23	5.14	5.61

Source: Adopted from Ennis et al. [181].

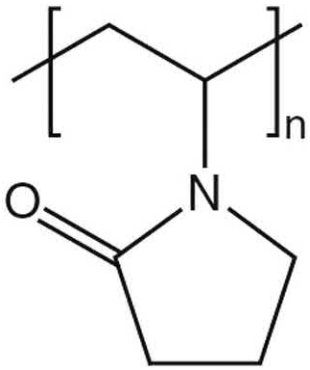

FIGURE 34.16 Chemical structure of the polyvinylpyrrolidone (PVP). (From Kozuka et al. [501].)

and hydrophilicity. Caseins do not coagulate by heat since they are very heat-stable proteins. Caseins are insoluble at their isoelectric point around pH 4.6. Furthermore, the solubility behavior of casein fractions isolated from milk is different. Generally, caseins are manufactured from skim milk by destabilizing the micelles [43].

34.2.5.3.2 Whey Proteins

Whey is a by-product of cheese or casein production and has some commercial uses. Whey proteins primarily include α-lactalbumin, β-lactoglobulin, serum albumin, and immunoglobulins (Table 34.6). Whey proteins are globular proteins and soluble in the ionic environment of milk, almost independent of pH [182]. However, they become insoluble at their isoelectric point and at low ionic strength (pH of about 5). Whey proteins become insoluble and denature at temperatures above 70°C and form thermally irreversible gels [183].

The remains after removal of caseins and fat from milk are whey proteins. There are different whey protein products that can be isolated, namely, whey powders of different quality (delactosed, demineralized, and demineralized–delactosed), whey protein isolate (WPI), whey protein concentrate (WPC), lactalbumin, and individual whey protein fractions. WPC typically contains 35–80% protein and a low level of fat. During the isolation of WPI, fat and lactose are removed yielding a higher protein content of >90%. The most important whey protein is β-lactoglobulin [183]. Proteins recovered from milk are becoming increasingly important, especially when considering emerging technologies, such as the microencapsulation of ingredients [184], and manufacture of edible films [185], which may benefit from further developments in this area [181].

34.2.6 Others

34.2.6.1 Polyvinylpyrrolidone (PVP)

Polyvinylpyrrolidone (PVP), 1-ethenyl-2-pyrrolidoinone homopolymer (Figure 34.16) is a synthetic neutral polymer, with a molar mass of about 1×10^4 to 5×10^5 g/mol. Powder of PVP is well soluble in both organic solvents and water. The good film-forming ability makes it a better polymer for coatings that requires temperature stability [186].

34.2.6.2 Shellac

Shellac is secreted by the lac insect *Laccifer lacca* found in some of the forests of northeast India, China, Bangladesh, and Thailand. Shellac is soluble in alkaline media such as sodium hydroxide, sodium carbonate, and ammonia as well as in several organic solvents. The chemical composition of shellac is still unknown, and its physical properties and composition depend on source and time of the harvest. For instance, its melting point ranges from 77°C to 120°C. Shellac is soluble in methanol, ethanol, glycol ethers, and glycols [187].

TABLE 34.6
Some Typical Characteristics of Whey Protein Fractions of Bovine Milk

Parameter	Whey Proteins			
	α-Lactalbumin	β-Lactoglobulin	Serum Albumin	Immunoglobulins
Concentration (wt%)	0.07–0.15	0.2–0.4	0.01–0.04	0.06–0.1
Molar mass ($\times 10^{-4}$ g/mol)	1.4	1.8	6.6	15–90
Isoionic point	4.2–4.5	5.2	5.3	

Source: Adopted from Ennis et al. [181].

34.2.6.3 Paraffin

Paraffin is the family of linear hydrocarbons with the general formula of C_nH_{2n+2}. It is dependent on the number of carbon atoms that could differ from 1 to 40. Paraffin is edible but not digestible, and is a white, tasteless, and odorless waxy material with a melting point ranging from 48°C to 95°C [43].

34.2.6.4 Inorganic Materials

There are some food-grade inorganic materials that have been described as being useful for microencapsulation or coatings in food applications, which include aluminum oxides, tripolyphosphate, or silicon oxides. They can be utilized alone or in combination with other materials [188, 189].

34.3 ENCAPSULATION

The food industry employs the encapsulation process for different reasons [190]; these include the following.

1) Encapsulation can protect the material from degradation and reduce interaction with the outside environment such as moisture, heat, light, and air, which may influence the ingredients and lead to loss of their functionality [20, 191].
2) Encapsulation can be used as a delivery system whereby the ingredient is released at the required time and at a certain point such as releasing of the leavening agent at a certain temperature during baking [20, 191].
3) The physical characteristics of the original material could be modified in order to allow its easy handling [20].
4) It could be used to separate components within a mixture that may react with one another [20].

34.3.1 Microencapsulation

Nearly 80 years ago microencapsulation was developed as a technology for packaging liquids, solids, or gaseous materials that are miniature or smaller than the normal size of the package [19, 192]. Designing of a microencapsulated ingredient requires knowledge of several factors: the core, the encapsulation material, interaction between the matrix or the wall material, the core and the environment, and the stability of the microencapsulated ingredient during storage. Although many ingredients are suitable to be encapsulated, processing costs are expensive and scaling up of the process might not always be feasible [193, 194]. The different methods for microencapsulation are described in the following sections.

34.3.1.1 Spray Drying

Spray drying is a low-cost technology commonly used at the industrial level. It offers the advantage of producing microcapsules in a fairly simple and economical way compared to other techniques [195]. Encapsulation by spray drying starts with homogenizing the active substance with the carrier material in a liquid medium (Figure 34.17). The emulsion is pumped into the drying chamber through a nozzle. At the exit of the nozzle tip, the droplets are allowed to come into contact with the drying fluid (hot gas) inside the drying chamber. Water is evaporated by hot air and a dry crust is formed on the droplet surface when the droplet water content reaches a critical value, and based on this process the wall material covers the active material. Finally, the dry microcapsules are collected at the powder collector of the cyclone or at the bottom of the dryer [196, 197].

Spray drying accounts for the majority of commercial encapsulated materials in food products [41]. It is typically used for the preparation of dry, stable food additives and flavors. At first, it is by no means obvious that spray drying is suitable for encapsulating flavors, because in principle the volatile flavor compounds evaporate faster than water. It is only a question of finding suitable carriers that prevent the volatile flavor compounds from being lost during the drying process, yet simultaneously allow water to evaporate unhindered [197]. Spray drying is economical, flexible in that it offers substantial variation in the encapsulation matrix, adaptable to commonly used processing equipment, and produces particles of good quality [198–200]. In fact, spray drying production costs are lower than those associated with most other

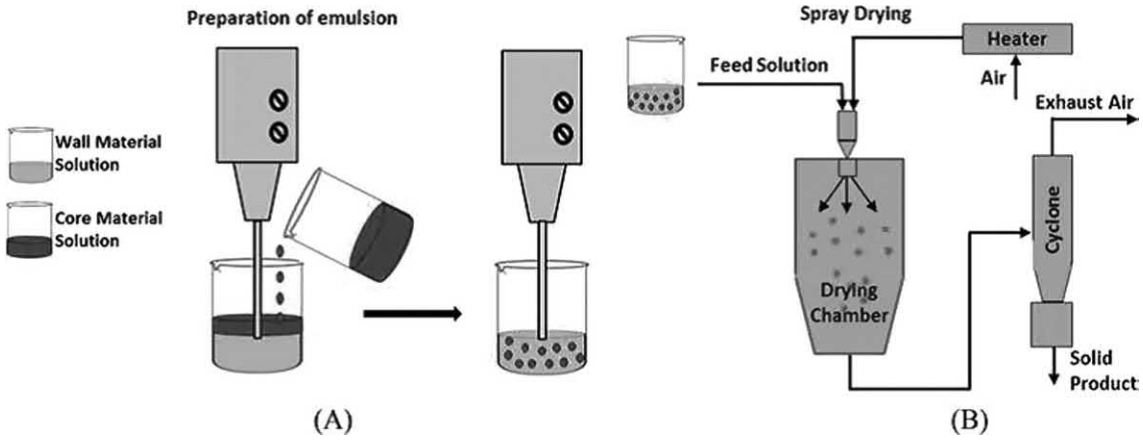

FIGURE 34.17 (A) Preparation. (B) Spray drying apparatus. (Adapted from Drosou et al. [197].)

methods of encapsulation. It is also one of the oldest and well-known encapsulation techniques—the C.E. Rogers Co. has been manufacturing spray dryers since the early 1900s— and is a standard technology in the food industry, especially the dairy industry [201]. In the 1930s spray drying was employed to prepare the first encapsulated flavors using gum acacia as the coating [202].

Although spray drying is most often considered as a dehydration process and is used in the preparation of dried materials such as powdered milk, it can be used as an encapsulation process when it entraps "active" materials within a protective matrix formed from a polymer or melt. In most cases, the encapsulated actives are released upon contact of the product with water, which dissolves the spray dried capsules [41]. The process is conducted in a spray dryer such as the one shown in Figure 34.17 and involves the following basic steps.

34.3.1.1.1 Preparation of the Dispersion or Emulsion

The initial step in spray drying an encapsulated food ingredient is the selection of a suitable wall material or encapsulating agent. The ideal choice should have adequate emulsifying properties; be a good film former; have low viscosity at high solids levels (<500 cps at ≥45% solids levels); exhibit low hygroscopicity; release the coated ingredients when reconstituted in a finished food product; be low in cost, bland in taste, and stable in supply; and afford good protection to the encapsulated ingredients [59, 203]. A food-grade hydrocolloid such as a gelatine, vegetable gum, modified starch, dextrin, or nongelling protein [38] is generally used as an encapsulating agent.

Once a wall material or combination has been selected, it must be hydrated. It is desirable to use a particular infeed solids level, which is optimum for each encapsulating agent or the combination chosen. Research has shown that the infeed solids level is the most important determinant of flavor retention during the spray drying process [48]. Increasing the solids level up to the point that the additional solids are no longer soluble benefits flavor retention by decreasing the required drying time to form a high solids surface film around the drying droplets. Once the droplet surface reaches about 10% moisture, flavor molecules cannot diffuse through this surface film, while the relatively smaller water molecules continue to do so and are lost to the drying air [204–207].

A high infeed solid means that this semipermeable membrane forms quickly and thus assists flavor retention. It is possible to pump and atomize infeed materials that contain encapsulating agent solids in excess of the solubility limits. Insoluble solids offer no barrier to the diffusion of flavor molecules and therefore do not improve flavor retention during drying. It has been found that there is an optimum infeed solids level that is unique for each wall material [203, 208, 209].

Once the encapsulating agent or mixture has been solubilized (with or without heating), the flavor or ingredient to be encapsulated is added to the mixture and then thoroughly dispersed into the system. A typical ratio of encapsulating agent to core material is 4:1, but in some applications higher flavor loads are used. Brenner et al. [210] have obtained a patent for a process that produces high-load spray dried flavorings. They claim that high surface oils and poor flavor retention during drying are largely due to particle shrinkage and cracking during the dehydration process. A cracked particle surface results in substantial flavor loss during drying. Brenner et al. [210] used a combination of polysaccharides (e.g., gum arabic, starch derivatives, and dextrinized and hydrolyzed starches) and polyhydroxy compounds (e.g., sugar alcohols, lactones, monoethers, and acetals) to form an encapsulating mixture, which remained in plastic during spray drying. Using this plastic encapsulating agent, Brenner et al. [210] reported to have spray dried infeed materials with a flavor load of up to 75% (based on dry solids). Mass balance data showed oil recoveries of 80% at this high loading. However, higher flavor loads typically result in an unacceptable loss of flavors in the dryer. For example, Emberger [211] has shown that compared to a 10% loading only 33% to 50% of the flavor was retained during drying when a 25% flavor load was used.

34.3.1.1.2 Homogenization of the Dispersion

Prior to spray drying, the mixture is homogenized in order to create small droplets of flavor or ingredient within the encapsulating solution. The creation of a finer emulsion increases the retention of flavor during the drying process [23]. Sometimes the addition of an emulsifier is required, and the dispersion is then homogenized prior to spray drying. However, considerable process variation exists within the industry in this respect. Risch and Reineccius [212] reported a direct relationship between the degree of homogenization and the retention of orange peel oil during spray drying. Therefore, it appears advantageous to efficiently homogenize the dryer infeed material. Water-soluble materials may also be encapsulated by the treatment of homogenization. Instead of having a clearly defined core and coating, the product consists of a homogeneously blended matrix of the polymer entrapping the core. These products are sometimes described as matrix particles or entrapped ingredients. They are also said to be covered with a very fine film of coating.

34.3.1.1.3 Atomization of the Infeed Emulsion

The core/wall material mixture is fed into a spray dryer where it is atomized through a nozzle or spinning wheel. The single-fluid, high-pressure spray nozzle and the centrifugal wheel are two types of atomizers that are widely used; the industry is nearly equally divided between their use. While each type of atomizer has its advantages and disadvantages, there is nothing in the literature that suggests that one type is superior to the other.

Atomization parameters have a significant effect upon the particle size distribution of the resultant powders. Several researchers have reported that larger particles result in improved flavor retention, but Reineccius and Coulter [46] found that particle size had no effect on flavor retention. On the other hand, studies by Chang et al. [213] indicated that there is an optimum particle size for flavor retention. Part of the controversy was cleared up by Bomben et al. [207] who showed that particle size was insignificant if high infeed solids were used. This might explain why some authors found a

relationship between particle size and flavor retention while others did not. Although particle size may have a minimal influence on flavor retention during drying, it is often desirable to produce large particles to aid in dispersion upon reconstitution. Small particles are often difficult to disperse and tend to float on liquid surfaces. Larger particles can be obtained by using a large orifice, low atomization pressure (pressure nozzle only), high infeed solids, high infeed viscosity, low wheel speed (centrifugal wheel atomization only), or some type of agglomeration technique [214].

34.3.1.1.4 Dehydration of the Atomized Particles

When hot air flowing in either a cocurrent or countercurrent direction contacts the atomized particles, water is evaporated and a dried product consisting of starch or an encapsulating matrix containing small droplets of flavor or core is formed. As the atomized particles fall through the gaseous medium, they assume a spherical shape with the oil encased in the aqueous phase. This explains why most spray dried particles are water-soluble. The rapid evaporation of water from the coating during its solidification keeps the core temperature below 100°C in spite of the high temperatures used in the process [215]. The particles' exposure to heat is in the range of a few seconds at most [38]. Thus, the main advantage to this method is its ability to handle many heat-labile materials. Because a flavor may contain as many as 20 to 30 different components (alcohols, aldehydes, esters, ketones, etc.) with boiling points ranging from 38°C to 180°C, it is therefore possible to lose certain low-boiling point aromatics during the drying process [199]. The dried particles fall to the bottom of the dryer and are collected, or they may be separated by a gas–solid separation unit such as a dust cyclone. Spray dried ingredients typically have a very small particle size (generally less than 100 μm), which makes them highly soluble but may present separation problems in dry blends. Separation can be prevented, and fluidity improved by a separate compacting or agglomeration step; in the latter, encapsulated particles are treated with steam to induce their cohesion and form large particles. Factors such as the coating structure may also affect the solubility of the spray dried microcapsules [216].

The processes of compacting and agglomeration complement spray drying. In both processes the objective, as stated above, is to obtain larger particles. Compacting gives a compressed product with lower porosity (strength). For example, spray dried flavors are compressed under high pressure into lumps and then crushed into small pieces ranging in size from 0.7 to 3.0 mm. This process is useful for applications where a grainy structure is required to assure that flavors will not separate (e.g., in tea bags). Agglomeration products, on the other hand, are loose products with high porosity (instant properties). Spray dried product is fluidized in hot air. The fluidization singles out powder particulates and allows them to be sprayed from all sides. By spraying onto a binder, such as water, the powder particles gradually stick to one another forming larger particles [198].

Feed temperature is one of the main factors that affect encapsulation efficiency and particle morphology. Feed temperature modifies the viscosity and homogeneity, therefore if feed temperature increases to a certain level, the droplet size and viscosity will decrease. However, extremely high temperatures could cause degradation or volatilization of some ingredients [217]. Shu et al. [218] encapsulated lycopene by using a gelatin–sucrose system and found that encapsulation efficiency increased through increasing feed temperature from 35°C to 55°C, while at 65°C encapsulation efficiency decreased. Brückner et al. [219] reported that the ideal feed temperature for microencapsulation of food ingredients was about 40°C because of the higher drying rate and the lower feed viscosity; however, a feed temperature of around 60°C was disadvantageous because it lowered the viscosity of the carrier material. Moreover, Yamamoto et al. [220] investigated the influence of feed temperature on encapsulation efficiency of D-limonene by using a mixture of gum arabic and maltodextrin. The results presented showed that the powder prepared at a higher temperature had better stability against the oxidation and release of D-limonene was higher than that of the powder obtained at a lower temperature. The findings might be partly attributed to an increase in shell thickness of the particle at higher temperatures, which creates a stronger barrier against the diffusion of oxygen as well as moisture from the surrounding environment. Furthermore, another study reported that the encapsulation efficiency of D-limonene was not significantly dependent on the feed temperature [221].

Another important parameter in spray drying is air inlet temperature and flow rate during the process, therefore these variables must be adjusted so that the liquid sprayed evaporates before it reaches the drying chamber. According to a recent study, powders with higher content of bioactive compounds are produced at lower inlet drying temperatures as well as a higher feed rate [222]. The encapsulation efficiency of lycopene, with maltodextrin as wall material, increased when inlet air temperature was increased from 110°C to 150°C, while the efficiency became almost constant when the temperature reached 160°C [223]. With an inlet temperature increasing from 170°C to 210°C, the encapsulation efficiency of lycopene increased at the beginning; however, it decreased dramatically when temperature reached 210°C [218]. Microencapsulation of vitamin C by using gum arabic as the wall material was studied at air temperatures varying from 140°C to 200°C and the wall material concentration of 10–20% [218]. Paramita et al. [221] found a reduction in flavor of spray dried powder product after increasing the inlet air temperature from 120°C to 150°C. In addition, they reported that higher inlet air temperature enhanced the vaporization of flavor compounds during the drying process.

The concentration of wall material in the solution can be increased in order to obtain a high microencapsulation efficiency, which could be related to the formation of a shell around the core material [224]. Rubilar et al. [225] reported that the microencapsulation efficiency of flaxseed oil increased from 54.6% to 90.7% after using a high concentration of 30% wall material that was composed of maltodextrin mixed with gum arabic instead of 25% wall material. Lewandowski et al. [226] spray dried nonfoamed and foamed emulsions of sunflower

oil and showed that the foaming process reduced the powder density by 50% compared to the powder produced from a nonfoamed emulsion in addition to increasing the microencapsulation efficiency.

Generally, wall materials have to be soluble in water in all spray drying processes in the food industry and must also have good emulsification properties, film forming around liquid droplets, and solutions of concentrated wall material should be of low viscosity [227, 228]. Flaxseed oil, one of the richest sources of plant-derived omega-3 fatty acids, has been successfully stabilized by spray drying. Sodium caseinate and whey proteins as wall materials improved the oxidative stability during storage of flaxseed oil [229].

In a recent study, the potential of a maltodextrin combination with different wall materials such as whey protein, gum arabic, and starch was examined for microencapsulation of flaxseed oil by using spray drying. The results showed that maltodextrin in combination with modified starches gave better encapsulation efficiency compared to a whey protein and gum arabic combination. Moreover, better emulsion stability and oxidation protection were found in the maltodextrin and whey protein combination [230].

There are certain factors that limit the use of spray drying. Firstly, the variety and number of wall materials available are limited, as they should have good water solubility. Secondly, the product considered as microcapsule powder needs additional processing like agglomeration. Another consideration is lower oxidative stability caused by the high temperatures used during the atomization process; this may limit the use of this technique [195]. For additional details about the spray drying process, the reader may view the reference by Drosou et al. [197].

34.3.1.2 Electrohydrodynamic Techniques

In these processes, electrostatic forces are used to produce electrically charged jets from viscoelastic solutions that on drying produce ultrathin structures by evaporating the solvent [231]. This technique is referred to as electrospinning when ultrathin continuous fibers are generated. Electrospraying consists of four main components: (i) a high-voltage source (1–30 kV), which is commonly operated in direct current mode, even though using alternating current mode is also possible; (ii) blunt-ended capillary or stainless-steel needle; (iii) a syringe pump; and (iv) a grounded collector (Figure 34.18) [232].

Both electrospinning and electrospraying work on the same principle with minor differences. The electrospinning process involves the application of a strong electrostatic field by the high voltage across a conductive capillary attached to a reservoir containing a polymeric liquid. Upon increasing the electrostatic field strength to a critical value, the solvent evaporates [233]. The difference between electrospraying and electrospinning, which might be considered as "sister" technologies, is based on viscosity and concentration of the polymer solution [234, 235]. The particle diameter or fiber and morphology in the electrospinning process are strongly dependent on several parameters [231] that include intrinsic properties of the polymer solution such as elasticity, viscosity, electrical conductivity, and type of polymer as well as operational conditions such as the strength of the applied electric field [236].

34.3.1.3 Spray Cooling and Spray Chilling

Spray cooling and chilling are two commercially practiced encapsulation processes similar to spray drying in that both involve dispersing the core material into a liquefied coating material and spraying through heated nozzles into a controlled environment [237]. Unlike spray drying, however, there is no water to be evaporated since the coating material is a fat. Other principal differences between these processes and spray drying lie firstly in the temperature of the air used in the drying chamber and secondly on the type of coating applied. Spray drying employs hot air to volatilize the solvent from a coating dispersion; in contrast, spray cooling and spray chilling use air-cooled to ambient or refrigerated temperatures. The core and lipid wall mixtures are atomized into the chilled air, which causes the fat to solidify around the core, thereby forming a crude encapsulated product. Spray cooling uses ambient or chilled air in order to solidify molten droplets, whereas spray drying uses heating of the feed line

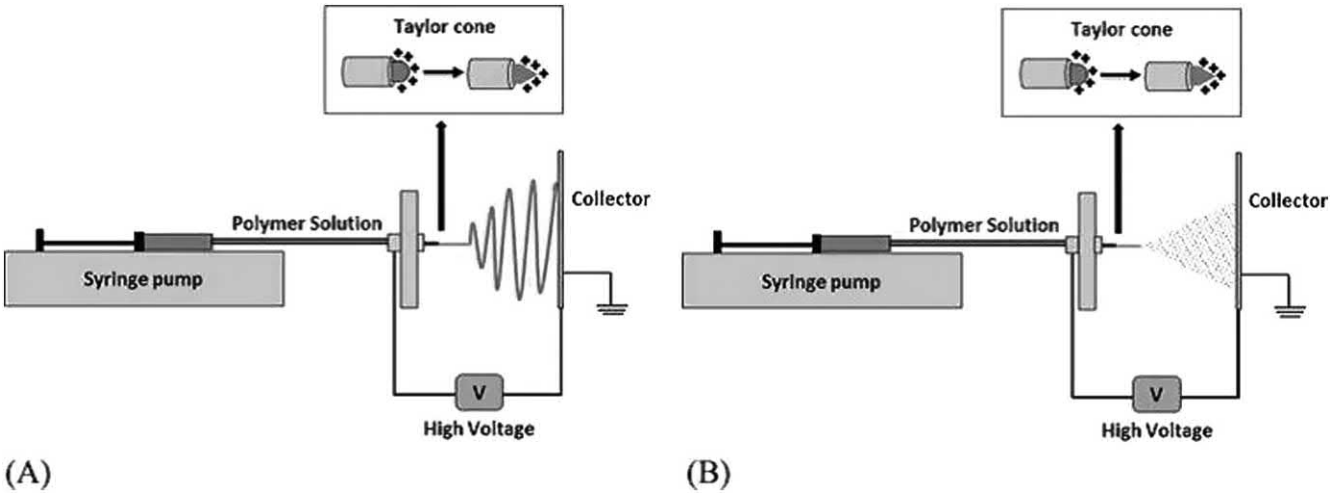

FIGURE 34.18 (A) Electrospinning. (B) Electrospraying. (From Drosou et al. [197].)

[238]. Spray cooling/chilling is a safe, rapid, and reproducible physical process. In addition, it is an environmentally friendly technique compared to other methods, such as spray drying. The most important aspect is the ease to scale up the production since this technique can be operated continuously [239].

In using this technique, the bioactive compound is dispersed or dissolved in a solution of the coating material. Furthermore, the vessel contains the cold medium, and as a result, the wall material solidifies forming a film coating. These techniques take place in one-step rapid process [240]. Spray cooling microencapsulation is considered the cheapest technology, uses lower temperatures and has a high potential for scale-up. It has been used to encapsulate both vitamins and minerals [241].

The particles produced are usually matrix type, and since this process does not include solvent evaporation, the particles produced by spray chilling are dense and mechanically resistant [242]. The spray cooling/chilling technique is cost-effective for probiotics [5] and also benefits from other advantages, as it does not include use of organic solvents that are lethal in the case of probiotics. Moreover, since the matrix is composed of fats, the release of bacteria occurs in the intestine due to the action of lipases [243].

Microcapsules produced by spray chilling and spray cooling are insoluble in water due to the hydrophobic coating. Consequently, these techniques tend to be utilized for encapsulating water-soluble core materials such as minerals, water-soluble vitamins, enzymes, acidulants, and some flavors. The major drawbacks of spray chilling include interactions between the fat and the active ingredient, volatilization of lipid-soluble materials over time, and loss of volatile materials during processing [41].

In spray cooling, the coating substance is typically a vegetable oil or one of its derivatives. Yet, a wide variety of other encapsulating materials can also be employed. These include fat and stearin with melting points of 45°C to 122°C as well as hard mono- and diacyl-glycerols with melting points of 45°C to 65°C. Taylor [199] indicated that mono- and diacylglycerols facilitate dispersion of the encapsulate in the finished, reconstituted food products and may also be considered as part of the overall emulsification system.

In spray chilling, the coating is typically a fractionated or hydrogenated vegetable oil with a melting point in the range of 32°C to 42°C. Coating materials with even lower melting points can be utilized, but their end products may require specialized handling and storage conditions [199]. Furthermore, in spray chilling there is no mass transfer, i.e., evaporation from the atomized droplets, and therefore the droplets solidify into almost perfect spheres to give free-flowing powders. Through atomization, it gives an enormous surface area, and an immediate and intimate mixing of these droplets with the cooling medium.

Spray chilling is used primarily for the encapsulation of solid food additives such as ferrous sulfate, acidulants, vitamins, minerals, and solid flavors, as well as for heat-sensitive materials or those that are not soluble in typical solvents [199]. With the functional food revolution, the importance of spray chilling has increased, particularly in the production of functional food ingredients for the primary purpose of fortification. Liquids may also be encapsulated following their conversion to a solid form, perhaps by freezing. The end products of the process, resembling fine beadlets of a large particle size, are water-soluble but release their contents at or around the melting point of the wall material. With the ability to select the melting point of the wall, this method of encapsulation can be used for controlled release. The process is thus suitable for protecting many water-soluble materials such as spray dried flavors that may otherwise be volatilized from a product during thermal processing. Spray chilled products have applications in bakery products, dry soup mixes, and foods containing high levels of fat [202].

Lamb [244] pointed out the importance of maintaining optimum temperatures during processing, as this can affect the fat's polymorphism, a phenomenon that describes the capability of a substance to exist in more than one crystalline form. He also noted that if a fat, say a powdered triacylglycerol, is permitted to exit from a chiller at a too high temperature, heat generated by polymorphism tends to reverse the encapsulating process and return the powder to a melt or perhaps a pasty mass.

De Lara Pedroso et al. [245] encapsulated probiotics using interesterified palm kernel and palm fat and found that the solid lipid microparticles provide protection for *Lactobacillus acidophilus* and *Bifidobacterium lactis* against the simulated gastric as well as intestinal fluids. Furthermore, promising results for viability during storage in both refrigerators and freezers were obtained. Okuro et al. [243] found similar results when encapsulating probiotics (*Lactobacillus acidophilus*) by using spray chilling with prebiotics in polydextrose within the lipid matrix. Solid lipid particles, prepared by spray chilling, are water insoluble because of the lipid carrier, which could limit some applications in food [190]. In general, these techniques are used for dry products in order to conserve all thermosensitive components such as vitamins, proteins, flavors, and minerals [246, 247].

34.3.1.4 Fluidized Bed Coating

Fluidized bed coating, also referred to as air suspension coating or the Wurster process, is a common technique used for commercial production of encapsulated ingredients for the food industry. It was developed by D.E. Wurster in the 1950s. In general, it has been found that dense particles with narrow particle size distribution and good flowability are most suitable for encapsulation by fluid bed. Ideally a particle size distribution between 50 and 500 μm is best, although it is possible to encapsulate particles ranging from 35 to 5000 μm [25].

Fluidized bed coating is considered as one of the most efficient coating methods with growing applications in the food as well as pharmaceutical industries. In this process, ingredients could be mixed, granulated, and also dried in the same vessel, therefore reducing the processing time and material handling compared to other processes [248]. Furthermore, fluidized bed coating is a powerful tool that produces microparticles in order to promote functionality of food products [249]. The

particles are suspended using an air stream at a specific temperature and sprayed with coating material. Evaporation of water can be controlled by some factors such as airflow, temperature, spraying rate, humidity of the air inlet, and water content [250].

Fluidized bed coating of food ingredients was once viewed as the last choice by a formulator on account of the costs involved. Today, however, high production volumes and well-developed technologies have made a number of encapsulated food products standard items and available at cost-effective prices [251]. Solid particles to be sprayed are suspended in an upward-moving column of air in a fluidized bed chamber at a controlled temperature and humidity. Depending upon the specific application, the airflow may be heated or cooled [237]. Once the moving fluid bed of particles has reached the prescribed temperature, the encapsulation coating material is introduced to the system. Great variations exist as to the type of wall material chosen. Cellulose derivatives, dextrins, emulsifiers, lipids, protein derivatives, and starch derivatives are examples of typical coating systems, and they may be used in a molten state or dissolved in an evaporable solvent. The coating is atomized through binary or pneumatic spray nozzles at the top of the chamber, whose droplets are of smaller size than the substrate being coated. The atomized particles travel down into the particle stream and deposit as a thin layer on the surface of suspended core material. The turbulence of the air column is sufficient to keep the coated particles suspended, thereby allowing them to tumble and become uniformly coated. Upon reaching the top of the air stream, the particles move into the outer, downward-moving column of air, which returns them to the fluidized bed with their coating nearly dried (Figure 34.19). The particles pass through the coating cycle many times per minute [37]. With each successive pass, the random orientation of the particles further ensures their uniform coating. In the case of hot melts, the coating hardens by solidification in cool air. In the case of solvent-based coatings, the coating is hardened by evaporation of the solvent in hot air. The amount of coating applied can be regulated by controlling the length of time (i.e., residence time) that the particles are in the chamber. In order to achieve a good degree of coating, the process takes anywhere from 2 to 12 h to complete. After this period, only 0.2–1.5% of the particles remains uncoated.

The amount of material that coats the particles depends on the length of time that the particles are in a chamber. The different methods of fluid bed include top spray, tangential spray, and bottom spray (Figure 34.19). In the top spray system, the coating solution is sprayed downward with air into the fluid bed that when porous particles move to the coating region they become microencapsulated. Opposing flows of the particles and coating materials lead to increased encapsulation efficiency as well as prevention of cluster formation. This system has been used successfully for obtaining very small microcapsules ranging between 2 and 100 μm. In addition, higher yields of microencapsulated particles were obtained compared to both tangential and bottom spray fluidized bed coaters [195].

Uncontrolled agglomeration of particles is the main problem in the fluidized bed process. In fact, the coalescence of the wet coated material occurs with the formation of liquid bridges between some particles [252]. The liquid bridges become solidified after evaporation of the solvent, thus forming agglomerates. Studies have shown that atomization must be controlled using some variables such as pressure, coating solution concentration, and inlet temperature in order to reduce agglomerations [253–255].

Fluidized bed spray granulation is a more recent technology that allows specific particle size distributions from 0.2 mm to 1.2 mm with low porosities to be designed into the product. As with spray drying, continuous spray granulation starts off with an aqueous emulsion. Repeatedly spraying, applying, and drying drops in a fluidized bed forms granules with an onion-like structure. A particular advantage offered by granulation technology is the possibility of producing large flavored particles of uniform particle size and shape without the need for any additional production steps [198].

The fluidized bed coating technique has been in use for a long time for the encapsulation of vitamins C and B and some minerals. Furthermore, encapsulates of calcium propionate and potassium sorbate produced by fluid bed coating

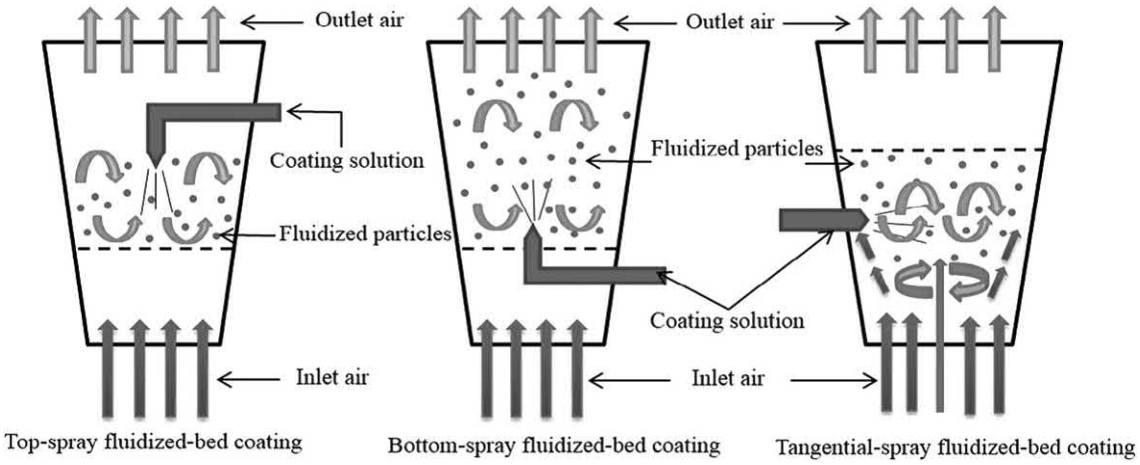

FIGURE 34.19 Diagram to show top, bottom, and tangential-spray fluidized bed coating. (From Bakry et al. [195].)

have wide applications in bakery products. In addition, some flavors and pigments are coated and used in the meat industry [251]. Because of equal temperature distribution, the fluidized bed technique could be carried out at significantly lower temperatures compared to other techniques such as spray drying. This is appropriate for the encapsulation of bacteria, where higher survival rates can be achieved [256, 257].

Schell and Beermann [258] found that the shellac coating of whey-encapsulated probiotic bacteria *Lactobacillus reuteri* might stabilize probiotics during both processing as well as storage. The coating also protects bacteria from acid conditions in the gastrointestinal tract and also improves the physiological effects of probiotics. The main point of selecting the right coating material (gelatine coating) between different types is to control release of fluidized-bed-coated menthol powder. Sixty percent of the menthol powder was released after about 11 min in water at 37°C [259]. Currently, the fluidized bed coating technique is used to encapsulate fish oil by spraying into the fluidized bed chamber followed by film-coating of the granules [248].

34.3.1.5 Extrusion

Encapsulation of food ingredients by extrusion is a newer process than spray drying. Extrusion, used in this context, is not the same as that used for cooking and texturizing of cereal-based products. The extrusion technologies for microencapsulation have been achieved using ram extrusion (melt injection) and screw extrusion (melt extrusion) [260–262]. Actually, extrusion, as applied to flavor encapsulation, is a relatively low-temperature entrapping method that involves forcing a core material dispersed in a molten carbohydrate mass through a series of dies into a bath of dehydrating liquid. The pressures and temperatures employed are typically <100 psi and seldom exceed 115°C, respectively [59]. Upon contact with the liquid, the coating material, which forms the encapsulating matrix, hardens and entraps the core material. Isopropyl alcohol is the most common liquid employed for the dehydration and hardening process. The extruded filaments or strands are then broken into small pieces, dried to mitigate hygroscopicity (an anticaking agent such as calcium triphosphate can facilitate this), and sized.

The main advantage of screw extrusion is its variation in terms of operating conditions, while the major disadvantage is the difficulty of accurately controlling the parameters of this complex setup [262, 263]. Co-extrusion comprises of a dual fluid stream of immiscible liquid core as well as shell materials. Both core and coating materials are pumped separately through the concentric feed tubes. Due to the action of surface tension, the wall material entraps the core material [264, 265].

Schultz et al. [266] were pioneers in the extrusion/encapsulation process. They emulsified orange peel oil in a molten dextrose mass, poured it on stainless steel sheets, and let it cool. The pulverized product exhibited good stability and flavor retention over a 6-month period. Combining the basic formulation of Schultz et al. [266] with extrusion, Swisher [267] created a novel encapsulating process that is similar to the one currently employed today by the flavor industry. The primary benefit claimed in his patent [268] was the maintenance of fresh flavor in encapsulated citrus oils, which otherwise would readily oxidize and yield objectionable off-flavors during storage. He conducted an accelerated shelf-life test on encapsulated orange peel oil that contained an antioxidant and found that its shelf life was about one year. Figure 34.20 shows the key steps for flavor encapsulation by extrusion.

In general, extrusion microencapsulation includes three processes: (i) melt injection, (ii) melt extrusion, and (iii) centrifugal extrusion (co-extrusion) (Figure 34.21). In the melt injection process, the core material is dispersed in molten carbohydrate and after that pressed through either one or more dies orifices to a bath of cold dehydrating liquid such as liquid nitrogen and isopropanol. The wall material solidifies with liquid forming an encapsulating matrix to entrap the core material. The granules are then recovered by centrifugation followed by using vacuum or air drying to remove any residual solvent. The melt extrusion process is also similar to the melt injection. However, the main differences are that melt injection is a vertical screwless process with surface-washed particles, whereas melt extrusion is a horizontal screw process with particles that are not surface-washed. Extruders used in melt extrusion are in a cylinder that contains thermomechanical mixers that consist of either one or more screws [195].

Centrifugal extrusion (co-extrusion) consists of a concentric feed tube through which core materials and wall materials are pumped separately to multinozzles mounted on the outer surface of the device. While wall material flows through the outer tube, the core material flows through the center tube [19]. There are several reasons extrusion encapsulation is popular in the food industry. It is easy to control the inlet temperature, which prevents degradation of heat-sensitive ingredients in addition to controlling the shape of the extruded product. The flexibility to control the processing conditions like moisture content, temperature, and screw speed is another advantage. Finally, extrusion is economical [15].

Swisher [268] added an essential oil such as orange peel oil, containing an antioxidant and a dispersing agent, to an aqueous melt of corn syrup solids (42 DE) and glycerine. The core syrup melt contained 3% to 8.5% moisture and was held at a temperature ranging from 85°C to 125°C, typically 120°C. The flavor/core syrup mixture was vigorously agitated while blanketed under nitrogen to form an oxygen-free emulsion. This emulsion was forced through a die into a hot immiscible liquid (e.g., vegetable or mineral oils), which was then rapidly cooled or extruded into pellets and allowed to solidify. The hardened pellets or solid globules were ground to a desired particle size, washed with isopropanol to remove surface oil and then dried under vacuum to yield a free-flowing granular material containing 8–10% flavoring.

The extrusion process of encapsulation has remained largely unchanged since Swisher's patent [268]. Most research developments to date concern the composition of the material that forms the encapsulating matrix. For example, Beck [269] replaced the high-DE corn syrup solids with a combination of sucrose and maltodextrin; a melt consisting of about 55% sucrose and 41% maltodextrin (10–13 DE). Even though the

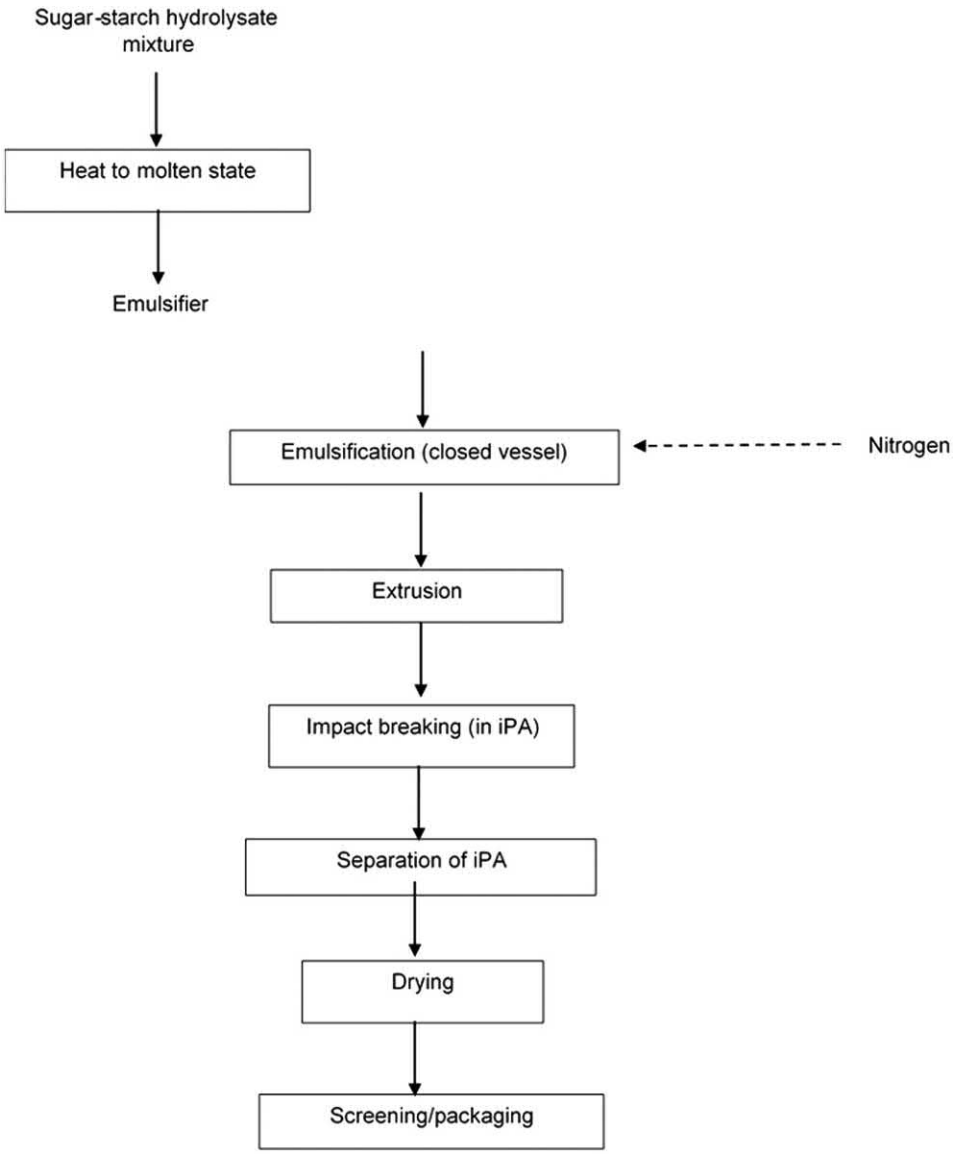

FIGURE 34.20 Flow diagram of encapsulation of food flavors via extrusion processing. (From Reineccius [47].)

low DE maltodextrin/sucrose matrix was considerably less hygroscopic than that used by some researchers [267, 268], Beck [269] continued to employ an anticaking agent, and even recommended pyrogenic silica rather than tricalcium phosphate. The flavor load obtained by Beck [269] ranged from 8% to 10%, with 12% considered as a practical maximum.

Barnes and Steinke [270] were awarded a patent for developing a modified food starch in place of sucrose in a similar process. Because chemically modified starches can possess good emulsification properties, the authors hypothesized that an emulsifying starch, with its lipophilic characteristics, would absorb the flavor oils into the matrix. The maltodextrin was, therefore, used primarily to provide bulk and some viscosity control. Barnes and Steinke [270] claimed that the use of emulsifying starches in the encapsulation matrix would permit increasing the loading capacity up to 40% flavoring.

Another benefit cited by the authors was that the total replacement of sucrose with emulsifying starches resulted in a product that was "sugar-free." This might have some advantages in the marketing of a final food product. Sucrose substituted with modified starches also provided greater flexibility to manufacturers. Because sucrose will invert to glucose and fructose at low pHs and high temperatures, the resulting product would be more hygroscopic and readily participate in nonenzymatic browning reactions. Therefore, the replacement of sucrose permitted longer cooking times, larger bath sizes, and higher cooking temperatures. Barnes and Steinke [270] also claimed that fruit juices, fruit essences, volatile substances, and propylene glycol could be encapsulated in this way using their encapsulation matrix. In order to successfully encapsulate fruit essences, it was first necessary to remove water and low-molecular-weight alcohols from the essence. The essence was then incorporated into an edible oil so that it would form an emulsion with the encapsulation matrix. For example, orange juice concentrate (42% water) could be encapsulated at 10–15% loading levels with their process. This was a

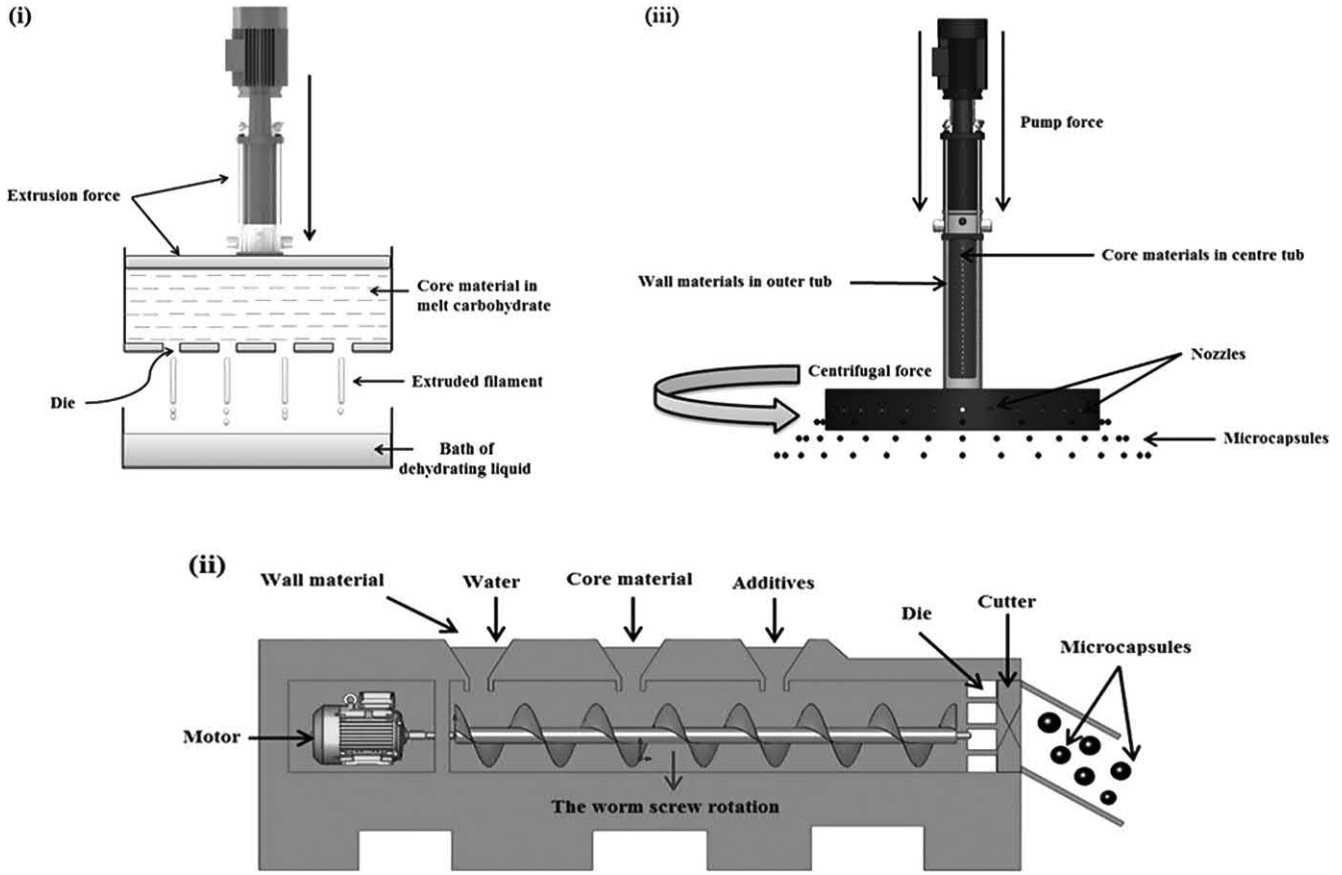

FIGURE 34.21 Diagram of microencapsulation by (i) melt injection, (ii) melt extrusion, and (iii) centrifugal extrusion (coextrusion) process. (From Bakry et al. [195].)

substantial improvement considering that prior formulations using sucrose were limited to 5–6% juice solids loading and could only be used with concentrates containing <20% water.

Miller and Mutka [271, 272] were awarded two patents for flavor encapsulation via extrusion. The first patent [271] involved a process for the encapsulation of orange juice solids, while the second dealt primarily with optimization of the extrusion process. It was their intent to improve the flavor load and encapsulation efficiency. A study of the effect of cooking temperature on flavor load and encapsulation efficiency indicated that high-load products (greater than 22%) had an optimum cooking temperature of about 123°C. As shown in Table 34.7, temperatures above or below this value yielded poorer encapsulation efficiencies. Because the cooking temperature is basically determined by moisture content, Miller and Mutka [272] postulated that too little moisture reduced emulsification effectiveness, while too much moisture hindered encapsulation. A cooking temperature of 123°C corresponded to ~5% moisture.

From the work of Miller and Mutka [272], optimization of cooking temperature, emulsifier concentration, and pressurization of the cooking vessel resulted in an improved encapsulation efficiency at high flavor loadings. Although their patent claims loadings of up to 35% can be used, only one example with loading as high as 27.6% was cited. The majority of examples demonstrated feasibility at flavor loadings from 15% to 20%, but still such levels are well over the traditional 8–10% flavor loadings achieved in commercial applications.

The extrusion process is particularly useful for heat-labile substances and has been used to encapsulate flavors, vitamin C, and colorants. According to Risch [22], extrusion provides true encapsulation in that the core material is completely surrounded by the wall material. When the material contacts the isopropanol and the wall is hardened, all residual oil or core material is removed from the surface. The absence of residual surface oil and the complete encapsulation gives products manufactured in this manner an excellent shelf life. This

TABLE 34.7
Influence of Cooking Temperature on Encapsulation Efficiency

Oil Encapsulated (%)	Encapsulation Efficiency (%)	Cooking Temperature (°C)
20.5	63.5	118
22.9	70.9	122
21.1	65.3	126
19.3	59.8	130
19.2	59.4	134

Source: Reineccius [47].

technique produces larger particles that can be used when visible flavor pieces are desirable. The primary advantage of extrusion is unquestionably its outstanding protection of the flavor against oxidation. For example, an accelerated shelf-life test on encapsulated orange peel oil containing no antioxidants was reported to be in excess of 4 years [267]. In terms of its weaknesses, extrusion is considerably more expensive than spray drying; process costs are estimated to be nearly double those of spray drying. Twenty percent flavor loading is standard for spray drying, while extrusion delivers less flavor per unit weight because its loading is currently running in the 8–12% range. Finally, one must realize that extrusion is a high-temperature batch process. The flavorings must be able to tolerate 110–120°C temperatures for a substantial period of time without deterioration.

Centrifugal extrusion is another encapsulation technique that has been investigated and used by some manufacturers. A number of food-approved coating systems have been formulated to encapsulate products such as flavorings, seasonings, and vitamins. These shell materials include gelatine, sodium alginate, carrageenan, starches, cellulose derivatives, gum acacia, fats/fatty acids, waxes, and polyethylene glycol.

Developed by scientists in the United States, centrifugal extrusion is a liquid co-extrusion process utilizing nozzles consisting of concentric orifices located on the outer circumference of a rotating cylinder (i.e., head) [273]. The encapsulating cylinder or head consists of a concentric feed tube through which coating and core materials are pumped separately to the many nozzles mounted on the outer surface of the device. While the core material passes through the center tube, coating material flows through the outer tube. The entire device is attached to a rotating shaft such that the head rotates around its vertical axis. As the head rotates, the core and coating materials are co-extruded through the concentric orifices of the nozzles as a fluid rod of core sheathed in coating material. Centrifugal force impels the rod outward, causing it to break into tiny particles. By the action of surface tension, the coating material envelops the core material, thus accomplishing encapsulation. The capsules are collected on a moving bed of fine-grained starch that cushions their impact and absorbs unwanted coating moisture. Particles produced by this method have diameters ranging from 150 to 2000 μm [274].

Another extrusion-based development is a process for encapsulating water-soluble lipids as particles of 1 to 15 mm. In this process, a core material is fed down a vertical tube while the coating material, a viscous solution of sodium alginate, simultaneously flows through a ring-shaped opening around the base of the tube, forming a membrane across the bottom of the device. The extruding core material pushes against the membrane until it eventually breaks off and carries a portion of the membrane with it. Upon spinning, the particles assume a spherical shape and become encapsulated. Passage through a bath of aqueous calcium acetate, calcium glutamate, or calcium lactate finishes this film-forming process by converting the coating to a water-insoluble calcium salt.

Chopde et al. [275] reported that the extrusion technique is an economical and simple method that is safe and does not cause damage to the probiotic cells and also affords high probiotic viability. Gouin [190] reported that the extrusion technique is primarily used for the encapsulation of unstable flavor and volatile compounds. Furthermore, this method is efficient for the encapsulation of phenolic compounds [276, 277].

Microencapsulation by extrusion is less used compared to spray drying. However, extrusion techniques have been used to encapsulate several essential oils including those of clove, olive, cinnamon, and thyme, for food industries, as well as pesticide industries [278, 279]. Sun-Waterhouse et al. [278] used this method to encapsulate olive oil by using alginate microspheres, and the study found that oil encapsulated by the co-extrusion technique was more stable during storage than unencapsulated oil.

34.3.1.6 Lyophilization or Freeze Drying

Lyophilization or the freeze drying method is a process for the dehydration of almost all heat-sensitive materials and aromas. It has been used to encapsulate water-soluble essences and natural aromas [280, 281] as well as drugs [282]. Except for the long dehydration period required (commonly 20 h), freeze drying is a simple technique that is particularly suitable for the encapsulation of aromatic materials.

Because the entire dehydration process is carried out at low temperature and low pressure, it is believed that the process should have a high retention of volatile compounds. Model system investigations by Thijssen and coworkers [206, 283] and Flink and Karel [45, 284] indicated that the retention of volatile compounds during lyophilization was dependent upon the chemical nature of the system; flavor retention increased when the molecular weight of the carbohydrate wall materials decreased and the level of total soluble solids increased (up to about 20%).

Freeze drying has long been known as the best drying method for maintaining the original properties of the product. It maintains the biological activity of aromas of food, flavors, and pharmaceuticals. Nevertheless, it is also an expensive method, about 4–7 times more expensive than spray drying [285], due to its long drying time and operation complexity [286]. There are three stages in the complete freeze drying process: freezing, primary drying, and secondary drying. First, the active compound is frozen and after that dried by sublimation under high vacuum [287]. The conditions such as low temperature and vacuum avoid oxidation and chemical modification of the products. This is the main reason that makes freeze drying an appropriate technology for products that are sensitive to heat [288]. For more details about these steps, see the reference by Liapis and Bruttini [289].

Many food and pharmaceutical companies use the freeze drying process in order to obtain dry products such as vaccines [290], viruses [291], proteins, peptides [292], and colloidal carriers [293]. Freeze drying can be used for dehydration of saffron with minimum crocin and safranal loss [294]. The same authors reported that the crocin and sarfanal contents of freeze dried samples were 5 times higher than the crocin and safranal contents of sun dried samples, which means that high cost of freeze drying could be compensated with a recovery of crocin and safranal.

For the production of citrus aroma powders to be used as natural flavor ingredients in soft drink dry mix formulations, Kopelman et al. [280] proposed the use of a freeze drying method. By simply dissolving various blends of corn syrup solids and sugars (mono- and disaccharides) in an aroma solution at a 25% (w/w) level followed by lyophilization, these authors claimed that approximately 75% of the initial aroma volatiles could be retained in the optimal maltodextrin sucrose mixture [280].

Freeze drying methods can also be used for other encapsulation processes. For example, Kirby and Gregoriadis [282] used freeze drying in the development of a technique known as dehydration–rehydration vesicles (DRV) for liposome entrapment. Upon the controlled addition of water, up to 70% of the water-soluble drugs present were entrapped into the formed liposomes. It has been reported that preparation of coatings only entrapped drugs that could be freeze dried again, and the liposomal structural integrity was apparently preserved. Intact liposomes with most of their contents still entrapped were obtained upon rehydration [158].

Indrawati et al. [295] encapsulated brown seaweed pigments using maltodextrin and Tween 80 by the freeze drying technique. The objective was to determine the stability of the active compounds that exist in the product upon encapsulation. In another study freeze drying was used successfully for microencapsulating several oils such as fish, walnut, flaxseed, and olive oils. The highest encapsulation yield (99.79%) was achieved for olive oil when carboxymethylcellulose, lecithin, and maltodextrin were used as encapsulants [296–299]. Furthermore, freeze drying has high consumption of energy (6–8 times) and requires longer times than spray drying. In addition, freeze drying renders a lower encapsulation efficiency [300].

34.3.1.7 Coacervation

The term *coacervation* originally originated from the Latin word *acervus*, which means "heap" [301]. Complex coacervation is a process that uses more than one encapsulating agent. In complex coacervation, electrostatic attraction is caused between at least two oppositely charged macromolecules. Furthermore, other weak interactions like hydrophobic interactions and hydrogen bonding may also play a role or contribute to the complex formation [302]. This technique is used to encapsulate different types of compounds such as vitamins, flavors, antioxidants, sweeteners, minerals, enzymes, fatty acids, and pre- and probiotics [303–305].

Coacervation is a phase separation phenomenon in which a homogeneous polymer solution is converted into two phases. One is a polymer-rich phase, called a coacervate, and the other is a polymer-poor phase, i.e., solvent [306]. Coacervation was developed and patented in the 1950s by the National Cash Register Company in the US and was used as a means of producing a two-component ink system for carbonless copy papers. Because of the very small particle sizes attainable with this process (ranging from a few submicrons to 6 mm), coacervation is regarded by many as the original and true microencapsulation technique [307].

The coacervation method is a commonly used encapsulation technique for some food applications for two reasons. First, it provides high encapsulation efficiency, and, second, because of the triggered controlled release that could be based on the temperature as well as providing the needed versatility to support many food products [190]. Jamekhorshid et al. [308] reported that the advantage of this method is its effectiveness in controlling particle size and its versatility. However, the disadvantages are high wall permeability and agglomeration. Figure 34.22 shows the flow diagram of a model complex coacervation technique that is commonly used for encapsulation of phase change materials (PCMs).

An example of a simple coacervation process could be described as follows: a gelling protein is dissolved in water, the core material is emulsified into the protein solution, and formation of the coacervate wall is initiated by changing either the temperature, pH, or by adding a concentrated salt solution. The resultant microcapsules are isolated by centrifugation or filtration. In effect, coacervation involves the separation of a liquid phase of coating material from a polymeric solution followed by the coating of that phase as a uniform layer around suspended core particles. The coating is then solidified. In general, batch-type simple coacervation processes consist of three steps, as summarised next, and are carried out under continuous agitation [36].

34.3.1.7.1 Formation of Three Immiscible Chemical Phase

In the first step, a three-phase system consisting of a liquid manufacturing vehicle phase, a core material phase, and a coating material phase is formed by either a direct addition or *in situ* separation technique. In the direct addition approach,

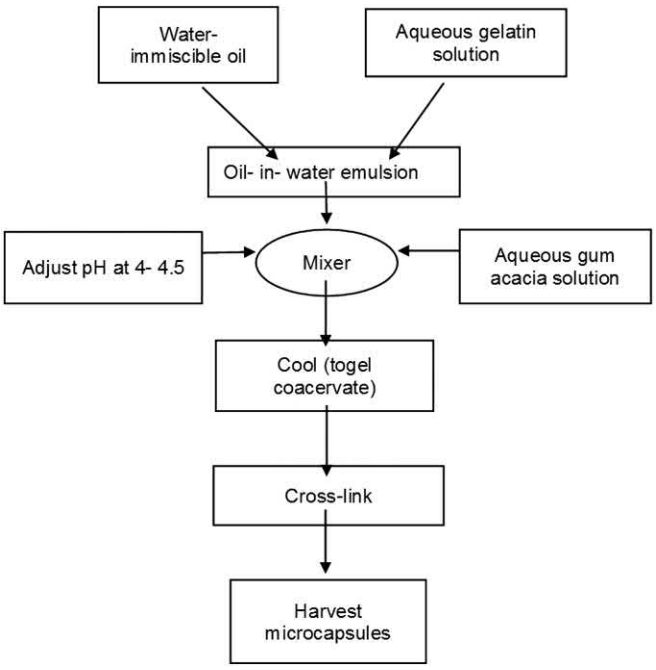

FIGURE 34.22 Flow diagram of a typical complex coacervation encapsulation process. (From Jamekhorshid et al. [308].)

the coating-insoluble waxes, immiscible polymer solutions, and insoluble liquid polymers are added directly to the liquid manufacturing vehicle, provided that it is immiscible with the other two phases and is capable of being liquefied. In the *in situ* separation technique, a monomer is dissolved in the liquid vehicle and then subsequently polymerized at the interface.

34.3.1.7.2 Deposition of the Coating

Deposition of the liquid polymer coating around the core material is accomplished by controlled physical mixing of the coating material (while liquid) and the core material in the manufacturing vehicle. Deposition of the liquid polymer coating around the core material occurs if the polymer is sorbed at the interface formed between the core material and the liquid vehicle phase; this sorption phenomenon is a prerequisite to effective coating. Continued deposition of the coating is promoted by a reduction in the total free interfacial energy of the system brought about by a decrease of the coating material surface area during coalescence of the liquid polymer droplets.

34.3.1.7.3 Solidification of the Coating

Solidification of the coating is achieved either by thermal, cross-linking, or desolventization techniques, and forms a self-sustaining microcapsule entity. The microcapsules are usually collected by filtration or centrifugation, washed with an appropriate solvent, and subsequently dried by standard techniques such as spray or fluidized bed drying to yield free-flowing, discrete particles.

Simple coacervation deals with systems containing only one colloidal solute (e.g., gelatine), while complex coacervation deals with systems containing more than one solute (e.g., gelatine and gum acacia [309] or gelatine and polysaccharide [310]). By strict definition, however, complex coacervation involves two biopolymers with opposite charges forming a complex coacervate as a result of ionic interaction at the interface. For example, positively charged type A gelatine (component A) forms complex coacervates with negatively charged polyphosphate (component B). Other systems that have been studied are gelatine/gum acacia, gelatine/pectin, gelatine/carboxymethyl guar gum, and whey protein/gum Arabic [306]. This technology has been used in flavor encapsulation as well as for the storage and delivery of additives [311]. The relatively high processing costs, sensitive multistep batch process, regulations limiting the number of polymeric agents that can be used in food preparations, and the difficulty in dealing with encapsulates having both aqueous and lipid solubility properties—as well as the sensitivity of these systems to high shear—have limited the application of coacervation for flavor encapsulation in the food industry [41].

Coacervation may also be subdivided into nonaqueous phase separation and aqueous phase separation techniques. Aqueous phase separation has been used to encapsulate citrus oils, vegetable oils, and vitamin A. It requires a hydrophilic coating, such as gelatine or gelatine–gum acacia, and water-insoluble core particles. The resulting microcapsules may contain payloads of 85% to 90% and can release their contents by pressure, hot water, or chemical reaction. For nonaqueous phase separation, the coating is usually hydrophobic, and the core may be water-soluble or water-immiscible. This process has been investigated for the encapsulation of solid food additives such as ferrous sulfate [38].

Although coacervation is very efficient, it can be an expensive process. It has found limited use in flavor encapsulation [24, 312], because of the high costs associated with the technology and difficulties encountered with the level of flavor that can be incorporated into the microcapsules [199]. Another reason cited by various industries for the limited use of coacervation is the problems associated with developing suitable encapsulating materials that are food approved. According to Blenford [202], the technology has been basically restricted to encapsulated ink systems used in carbonless office forms and to encapsulated fragrances that are applied in the form of scratch-and-sniff strips in promotional literature. Arneodo et al. [313] and King [314] observed that research into this technology was in progress and proposed that commercial applications would be available in the near future.

Ocean Nutrition Canada (ONC), now DSM, uses a proprietary microencapsulation technology based on a complex coacervation process to encapsulate fish oil concentrates rich in eicosahexenoic acid (EPA) and docosahexaenoic acid (DHA). The technology is based on the patent of Yan and Jin [306]. Research has suggested that a diet containing omega-3 fatty acids, such as EPA and DHA, may have a beneficial effect in lowering the incidence of cardiovascular disease [315]. Although there is no established daily requirement for the omega-3 fatty acids, recommendations range from 250 to 500 mg EPA/DHA per day. ONC's patented process involves preparing an emulsion comprised of fish oil and a gelatine-based polymer. The emulsion has a particle size of <100 mm. Two oppositely charged polymer colloids—one an amphoteric polymer and the other a polyanionic polymer—interact with each other to cause phase separation. The product is introduced into a spray drier and a dried microcapsule results. MEG-3™ powder is a patented microencapsulated fish oil product comprising a 60% payload of fish oil; this represents one of the highest, if not the highest, concentrations of bioavailable omega-3 in the market place. MEG-3™ powder is manufactured to withstand shear and high temperature and other food processing operations such as pasteurization and homogenization. Studies conducted by ONC have shown that there is no loss of omega-3 EPA/DHA during processing or via shelf-life deterioration. This functional food ingredient has been used to develop an omega-3 yogurt product. Delivering 50–100 mg of EPA/DHA per serving in a variety of functional foods will help consumers reach the recommended daily intake levels.

This method was found to be especially suitable for high-valued as well as sensitive functional ingredients such as polyphenols [18]. In another study, Zheng et al. [316] encapsulated anthocyanins using this method, which can be used to encapsulate heat-sensitive ingredients because it is done at room temperature.

34.3.1.8 Centrifugal Suspension Separation

Centrifugal suspension separation is a more recent microencapsulation process. The process has been patented [317,

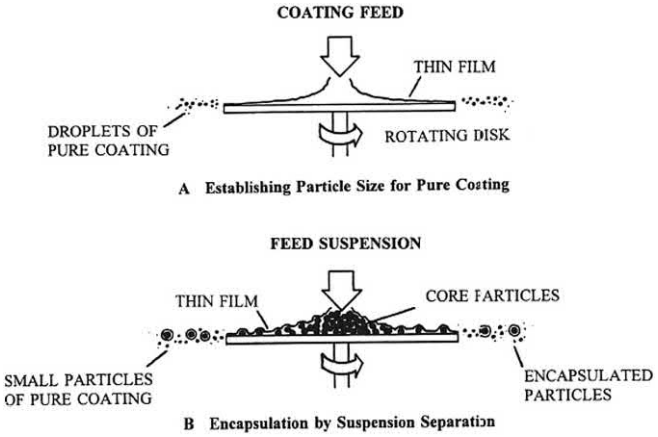

FIGURE 34.23 Representation of rotational suspension separating system. (From Sparks and Mason [317].)

318] and was first applied commercially in February 1987 to a chemical produced in Europe. The process, in principle, involves suspending core particles in a pure, liquefied coating material, and then pouring the suspension over a rotating disk apparatus under such conditions that excess liquid between the core particles spreads into a film thinner than the core's particle diameter. The excess liquid is atomized into tiny droplets, separated from the coated product and recycled. The core particles leave the disk with residual liquid still around them, which forms the coating. The particles are hardened by chilling and drying [319]. The principle behind this process is illustrated in Figure 34.23.

Centrifugal suspension separation is a continuous, high-capacity process that takes seconds to minutes to coat core particles. The process can handle a wide variety of core materials, including those that are temperature sensitive, and coating materials, in solid, liquid, or suspension states, without presenting aggregation problems. Furthermore, the process handles each particle only once and, under most conditions, produces no uncoated particles. The process has been used successfully to coat particles ranging from 30 μm to 2 mm. Coatings have been produced with thicknesses ranging from 1 to 200 μm. Microcapsules have been prepared with payloads ranging from 1% to 97%, depending on diameter size of the particle. Another advantage associated with centrifugal suspension separation is that the size distribution of the encapsulated particles resembles that of the uncoated particles.

34.3.1.9 Cocrystallization

Cocrystallization is an encapsulation process utilizing sucrose as a matrix for the incorporation of core materials. Although granulated sugar is composed of solid, dense, monoclinic spherical crystals with a limited surface area, it is not suitable as an encapsulating agent for flavor encapsulation. In order for flavors to be incorporated into the matrix, the structure of sucrose must be modified from a single perfect crystal to a microsized, irregular, agglomerated form, to increase void space and surface area [74, 320]. It involves spontaneous crystallization, which produces aggregates of micro- or fondant-size crystals ranging from 3 to 30 μm, while causing the inclusion of entrapment of all nonsucrose materials within or between sucrose crystals [321]. Use of the cocrystallization process allows many types of food ingredients, either single ingredients or combinations of ingredients, to be incorporated permanently into a crystalline sucrose aggregate, thus providing interesting and useful characteristics.

Sucrose syrup is concentrated to the supersaturated state and maintained at a temperature high enough to prevent crystallization. A predetermined amount of core material is then added to the concentrated syrup with vigorous mechanical agitation, thus providing nucleation for the sucrose/ingredient mixture to crystallize. As the syrup reaches the temperature at which transformation and crystallization begin, a substantial amount of heat is emitted. Agitation is continued in order to promote and extend transformation/crystallization until the agglomerates are discharged from the vessel. The encapsulated products are then dried to a desirable moisture (if necessary) and screened to a uniform size [80, 81]. It is very important to properly control the rates of nucleation and crystallization, as well as thermal balance during the various phases. The essential steps for the preparation of cocrystallized flavor are presented in Figure 34.24.

The agglomerates form a loose network, bonded together by point contacts. The encapsulated materials are located primarily in the interstices between crystals. Due to the porosity of the agglomerates, it is easy for an aqueous solution to rapidly penetrate the agglomerate and release the core materials for dispersion and/or dissolution.

The cocrystallization process offers several advantages, for example, it can also be employed to achieve particle drying. In the highly saturated solution, nucleation and crystallization proceed at a rapid rate and the resulting heat of crystallization can be used to affect particle dehydration by evaporation. By means of the cocrystallization process, core materials in a liquid form can be converted to a dry powdered form without additional drying. Because the flavor or core material is well entrenched in the modified sucrose matrix, there is no tendency for flavor material to separate from or settle out during handling, packaging, or storage. Additionally, all cocrystallized sugar/flavor products offer direct tableting characteristics because of their agglomerated structure, and thus offers significant advantages to the candy and pharmaceutical industries [322].

34.3.1.10 Liposome Entrapment

Liposomes provide an attractive encapsulation system for the delivery of both hydrophilic and lipophilic functional compounds. They are spherical-shell structures consisting of one or more phospholipid bilayers enclosing a liquid core [323]. Liposomes can be classified based on their lamellarity into three types, namely, oligolamellar vesicles (OLV), unilamellar vesicles (ULV), and multilamellar vesicles (MLV). Vesicles can likewise be categorized by their size as giant unilamellar vesicles (GUV), large unilamellar vesicles (LUV), and small unilamellar vesicles (SUV) (Figure 34.25) [324, 325]. A number of methods for liposome preparation are presently

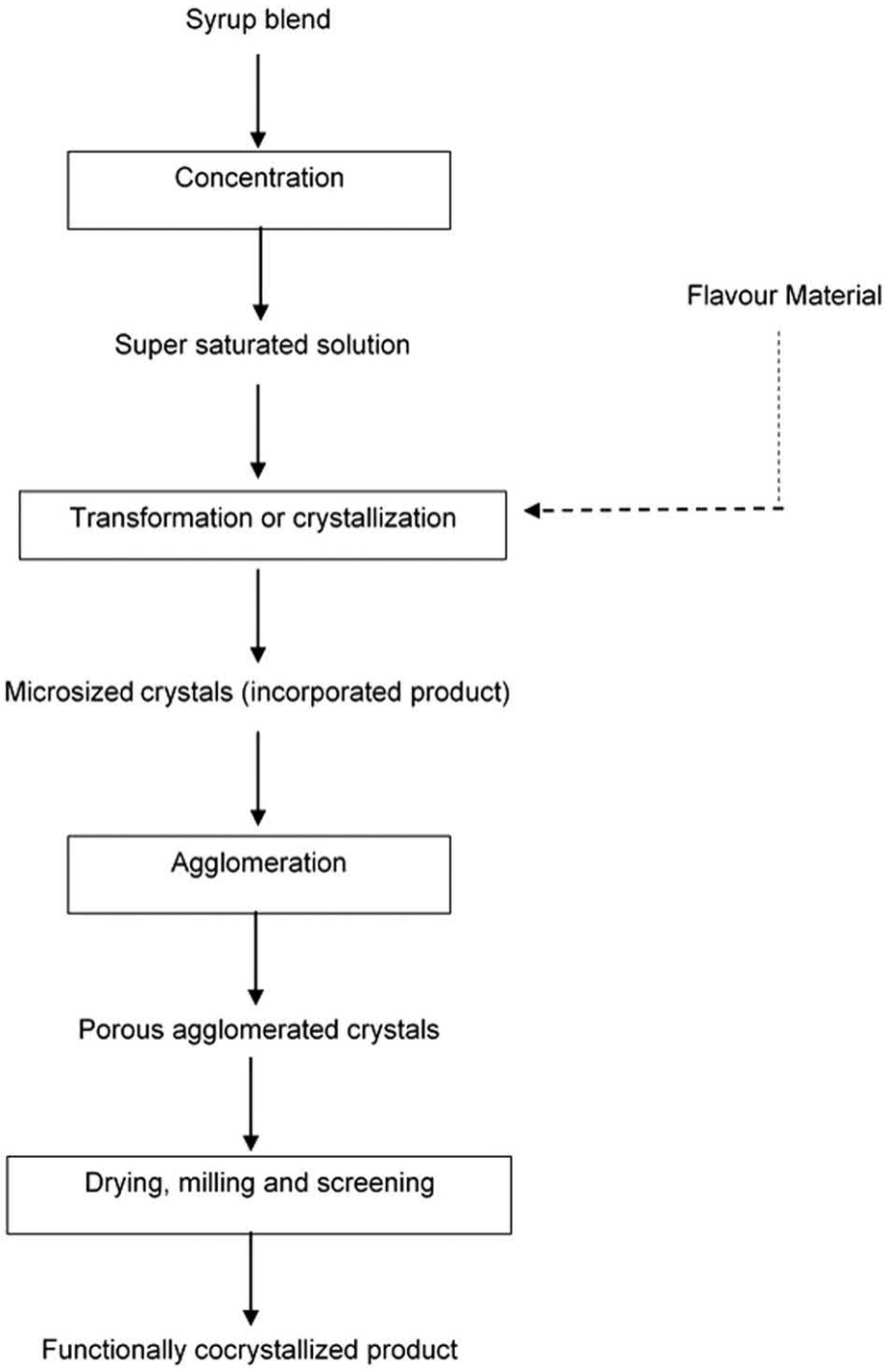

FIGURE 34.24 Essential steps for the preparation of a cocrystallized flavor. (From Chen et al. [74].)

available such as the solvent injection method, heating-based method, and thin-film hydration method [326].

Liposomes are usually used to deliver proteins [327], enzymes [328], vitamins [329], minerals [330], flavors [331], antioxidants [332, 333], and antimicrobials [334] in food products. A number of strategies are used for the preparation of liposomes. Further details about these preparation methods have been described elsewhere [323].

Liposomes provide a promising solution for protecting vitamins from degradation. Wechtersbach, Ulrih, and Cigić [335] reported that increased half-life of liposomal vitamin C was about 300 times in a model system and it also decreased the rate of oxidation in apple juice. However, liposomal stabilization of vitamins in fermented milk products was lower due to the integration of the lipids in the product into the membrane, therefore increasing liposome permeability and milk lipoprotein lipase, which may lead to hydrolysis of membrane phospholipids.

Numerous methods of liposome entrapment have been developed [156, 158, 336]. Preparations may vary widely

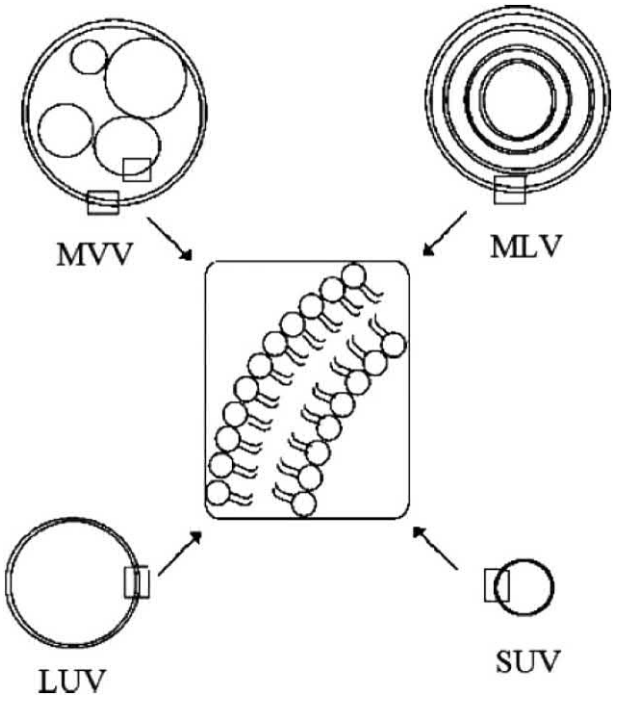

FIGURE 34.25 Liposome classification based on lamellarity and size. (From Emami et al. [323].).

in vesicle size distribution, number of bilayers per vesicle, and encapsulation efficiency. Liposomes consist of an aqueous phase that is completely surrounded by a phospholipid-based membrane. When phospholipids, such as lecithin, are dispersed in an aqueous phase, the liposomes form spontaneously. One can have either aqueous or lipid-soluble material enclosed in the liposome. However, liposome entrapment for many flavor compounds is not possible because liposomes will not form with materials that are soluble in both the aqueous and lipid phases [22]. From a physicochemical point of view, the formation of liposome structures may be illustrated by phase diagrams. A simplified phase diagram of the 1,2-dipalmitoyl phosphatidylcholine–water system is shown in Figure 34.26 [337]. The addition of water decreases the transition temperature of the phospholipid to a limiting value (T_c), which is the minimum temperature required for water to penetrate between the layers of lipid molecules. When the system is cooled below T_c, the hydrocarbon chains adopt an ordered packing. The structure of this phase, known as the gel, is lamellar and the hydrocarbon chains extended [337]. Each type of phospholipid molecule is characterized by a phase transition temperature. Below T_c, its fatty acyl chains are in a quasicrystalline array, while above T_c, the chains are in a fluid-like state.

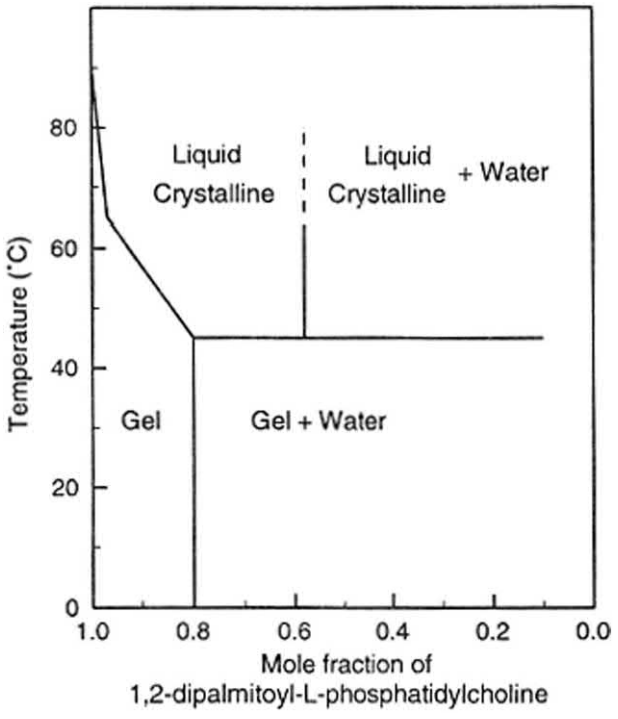

FIGURE 34.26 Phase diagram of the 1,2-dipalmitoyl-L-phospha tidylcholine-water system. (From Shahidi and Han [499].)

There are two principal requirements for liposome microencapsulation. Firstly, the lipid of choice must have a negative Gibb's free energy value (ΔG) for bilayer structure formation, because a negative ΔG value between two states of the system indicates a favorable reaction. Secondly, sufficient energy must be put into the system to overcome the energy barrier. Close to room temperature, the value of ΔG for the formation of liposomes is always negative and, therefore, favorable. Even though thermodynamics are favorable, this does not mean that the reaction will proceed automatically; it is usually necessary to overcome an energy barrier in order to initiate a reaction. Different lipids and types of energy input may be used to produce different varieties of liposomes for specific purposes. Some methods commonly employed are described next.

34.3.1.10.1 Microfluidization

The microfluidization technique is based on the dynamic in specially designed microchannels. The resulting momentum and turbulence allow the lipid emulsion to overcome the energy barrier ($\Delta G^{\ddagger}$). An air-driven microfluidizer operates at pressures of up to 10,000 psi. A pump driven by compressed air is used to pump the aqueous emulsion of lipids, and the single-feed stream is split into two fluidized ones. The two flows interact with one another at ultrahigh velocities and in precisely defined microchannels.

Mayhew et al. [338, 339] found that small (0.1 μm in diameter) liposomes with high solute-capture efficiency could be easily formed by microfluidization technology. At an initial lipid concentration of 300 mM, up to 75% of cytosine arabinoside was captured in the aqueous space of these liposomes. Advantages of microfluidization include (i) a large volume of liposomes can be formed in a continuous and reproducible manner; (ii) the average size of the liposomes can be adjusted; (iii) very high capture efficiencies (larger than 75%) can be obtained; (iv) the solutes to be encapsulated are not exposed to sonication, detergents, or organic solvents; and (v) the resulting liposomes appear to be stable, and do not aggregate or fuse.

34.3.1.10.2 Ultrasonication

Ultrasonic dispersion is often used for the preparation of SUV; the lipid emulsion overcomes the energy barrier through ultrasound absorption. In one approach, phospholipids are sonicated by immersing a metal probe directly into a suspension of large liposomes. In a second method, the lipid dispersion is sealed in a glass vial, which is then suspended in an ultrasonic cleaning bath. Bath sonication requires longer periods (up to 2 h) than probe sonication (only a few minutes), but it has the advantage that it can be carried out in a closed container under nitrogen or argon and does not contaminate the lipid with metal from the probe tip [160].

34.3.1.10.3 Reverse-Phase Evaporation

The reverse-phase evaporation technique has been developed for the preparation of LUV in which lipids in mixed aqueous-nonpolar solvents form inverted micelles (i.e., the lipid tails are inserted into the nonpolar phase and the head groups surround water droplets). When the nonpolar solvent is removed by rotary evaporation under vacuum, the gel-like intermediate phase changes into large unilamellar and oligolamellar vesicles. This procedure produces liposomes of quite uniform size, ranging from 0.1 to 1.0 μm in diameter, with high encapsulation efficiency of up to 65% in low ionic strength media. However, its disadvantage is that components are exposed to both organic solvents and sonication. This may result in the denaturation of proteins and other molecules of similar stability [159].

34.3.1.11 Interfacial Polymerization

Interfacial polymerization happens when two different polymeric solutions are brought together. These two reactive polymeric species, each solubilized in a different liquid, react with one another when one liquid is dispersed in the other. The polymerization reaction takes place at the interface between the two polymeric liquids.

The interfacial polymerization process can be used to encapsulate solutions or dispersions of hydrophobic materials. It can also be used to encapsulate aqueous solutions or dispersions of hydrophilic substances. In the interfacial polymerization microencapsulation process, both the dispersed and continuous phases serve as a source of reactive polymeric species. In general, an interfacial polymerization reaction proceeds at a rapid rate that results in the formation of a very thin film having physical property characteristics of a semipermeable membrane. Properties of the film are markedly influenced by the reaction time [340].

The ultimate capsule size of interfacial polymerization is determined by the size of the first monomer. In general, the capsule size ranges from about 1 μm to several millimeters. This capsule size is a direct function of the agitation rate [340]. It was found that an increase in the concentration of the emulsifier yields a narrow size distribution range and a reduction of the average particle size. The patent application for the microencapsulation process utilizing the principle of interfacial polymerization was filed by IBM (serial no. 813,425) in 1959 [340]. However, use of interfacial polymerization for food systems is limited since most coatings are not food grade.

34.3.1.12 Inclusion Complexation: Molecular Inclusion

Molecular inclusion is another means of achieving encapsulation. Unlike other processes discussed to this point, this technique takes place at a molecular level and β-cyclodextrin is typically used as the encapsulating medium [61]. As previously noted, β-cyclodextrin is a cyclic glucose oligomer, consisting of seven β-D-glucopyranosyl units linked by α-(1⟶4) bonds. Due to its molecular structure, β-cyclodextrin has limited solubility, a hydrophobic center, and a relatively hydrophilic outer surface, all of which affect the compound's formation of complexes.

The β-cyclodextrin molecule forms inclusion complexes with compounds that can fit dimensionally into its central cavity. These complexes are formed in a reaction that takes place only in the presence of water. Molecules that are less polar than water (i.e., most flavor substances) and have suitable molecular dimensions to fit inside the cyclodextrin interior can be incorporated into the molecule. In aqueous solutions, the slightly nonpolar cyclodextrin interior is occupied by water molecules. This situation is energetically unfavorable and, therefore, the sites occupied by water are readily substituted by the less polar guest molecules. Cyclodextrin complexes are relatively stable and their solubility in aqueous solutions is reduced compared to the uncomplexed cyclodextrin. Therefore, the complexed cyclodextrins readily precipitate out of solution and can be recovered simply by filtration.

The complexing of a cyclodextrin with a guest compound can be accomplished by three methods [69]:

(i) Stirring or shaking the cyclodextrin and guest molecules to form a complex that then canbe easily filtered and dried. Although in some cases, complexation of an insoluble guest can only be accomplished through dissolution of the guest in a water-soluble solvent.
(ii) Blending of solid β-cyclodextrin and guest with water to form a paste. Solvent should not be used. This method is particularly applicable for oleoresins.
(iii) Forcing a gas through the solution for complexation to occur. This method is seldom used.

However, it should be emphasized that there are several variations to these basic techniques, but still in all methods both the cyclodextrin and the guest molecules must be solubilized. If the guest material is insoluble in water, it is necessary to dissolve it in another solvent such as alcohol.

The composition of the cyclodextrin complex formed depends greatly upon the molecular weight of the guest molecule in question. Because one molecule of cyclodextrin will normally include only one guest molecule, the loading depends upon the compounds included. It should be noted that the theoretical maximum loading is not always obtained. For example, Pagington [341] stated that dimethyl sulfide should be complexed at 5.5% but only 2% loading has been observed.

It has been reported that cyclodextrins have a variable affinity for different guest compounds. This may be used to the advantage, or it can be disadvantageous. Some researchers have made use of the variable-binding properties offered by β-cyclodextrin to selectively remove bitter compounds from orange and grapefruit juices [342]. Variable binding properties can also be a disadvantage when it comes to the encapsulation of flavor compounds. Reineccius and Risch [61] formed 0% (isoeugenol) to 100% inclusions (ethyl hexamoate and linalool) when they added a model flavor system to β-cyclodextrin in an ethanol:water mixture. The losses of flavor compounds were due to the lack of inclusion rather than a loss during the subsequent complex recovery and/or drying steps. Once the complex was formed, it was quite stable to evaporation.

The variable inclusion properties of cyclodextrins would result in a dry flavor quite different from that of the original flavor when the flavor is comprised of a broad range of flavor molecules (e.g., an artificial flavor that contains short-chain esters and longer-chain character impact compounds). However, flavors such as orange, which have been included in β-cyclodextrin, may not be distinguishable from fresh orange even by trained taste panels [343].

There are substantial data in the literature that document excellent protection for substances that have been treated with cyclodextrins [24, 343–345]. As previously mentioned, the cyclodextrin–guest complex formed is very stable to evaporation. Szente and Szejtli reported [345] only about a 5% loss of included volatiles after 2 years of storage at room temperature. However, more important is the oxidative stability of the included guest compounds. Many reports have demonstrated that inclusion complexes are quite stable to oxidation [343, 345].

As with all processes, there are limits to the application of cyclodextrin complexation in the formation of flavors [346]:

(i) There is a limited amount of flavor content in formulations (average 9% to 14% by weight).
(ii) The size and polarity of flavors to be complexed limits the usefulness of the process.
(iii) Cyclodextrin can act as an artificial enzyme, sometimes enhancing the rate of hydrolysis of some ester-type flavor components. This can result in the undesirable adulteration of the flavor.
(iv) The water solubility of β-cyclodextrin flavor complexes is generally much lower than that of spray dried and other microencapsulated samples.

34.3.2 Nanoencapsulation

The encapsulation of bioactive compounds at a size of 100–500 nm or less, usually at lower than 100 nm is known as "nanoencapulation" and also the material is called "nanomaterial" [347]. Nanoencapsulation is one of the most promising technologies that have the ability to protect and entrap bioactive compounds [14]. The two main reasons behind high and slow release and water solubility of nanoencapsulated materials compared to native counterparts are firstly due to the reduction in size of the particles to become nano, leading to increased surface area and hence bringing maximum active sites on the surface and augmenting their penetration. Secondly, the tailor-made carrier material controls the gradual release of bioactive compound and therefore allows maximum absorption in the digestive system [348–351]. With the employment of nanoencapsulation, deliveries of multiple active ingredients that do not normally mix well, such as water-soluble and lipid-soluble vitamins, can be released consecutively. The role of nanotechnology in delivery systems for bioactives and specialty gradients has advanced considerably in recent years [182].

34.3.2.1 Classification of Food Nanodelivery Systems

Recently, different types of nanodelivery systems have been developed and reviewed in the literature. These include nanostructured lipid carriers (NLCs); nanoemulsions; solid lipid nanoparticles (SLN); nanosuspensions; liposomes and nanoliposomes; polysaccharides; and combinations with lipid or mineral components, nanoparticles (NPs), and micelles made of proteins [352–355].

Nanoemulsions are colloidal dispersions of small liquid droplets, less than 100 nm (Figure 34.27a) [355, 356] and might be water-in-oil (W/O) or oil-in-water (O/W), and either liquid-in-liquid or liquid-in-solid such as butter and a W/O solid emulsion. The interface might be stabilized by different types of emulsifiers, in which their structure and amphiphilicity determine the size droplet curvature. In addition, the oil type and composition as well as the surfactant-to-oil ratio might affect emulsion stability and droplet size [357].

The major types of amphiphiles used for nanoemulsion formation include high molecular weight biopolymeric amphiphiles [358], either natural (such as milk proteins [359], milk protein peptides [360], whey proteins, mainly beta-lactoglobulin [361], and particularly caseins [362]) or synthetic (such as octenyl succinate modified starch [363]); and low molecular weight surfactants, either natural surfactants (such as saponins [364] and phospholipids [365]) or synthetic surfactants (such as Tween 20 [366], Tween 80 [367] and sucrose palmitate [368]).

Generally, nanoemulsions are made by using low-energy or high-shear/high-energy homogenization methods. In addition, spontaneous self-emulsification methods may be used [357, 369]. Nanostructured lipid carriers (NLCs) include partially crystallized lipid nanovehicle particles with a mean size of about 100 nm, which are dispersed in an aqueous phase containing emulsifiers (Figure 34.27c). The partially solid material creates nanostructures that promote stability of entrapped bioactives and increase loading capacity [370]. Another type of NLC is pickering emulsions (Figure 34.27d) in which solid NPs are adsorbed or sintered to the oil–water interface and also provide much higher stability to emulsions [354, 371]. Nanosuspensions are systems in which solid NPs are dispersed in a liquid medium (Figure 34.27e). The noanosuspension is normally made of nanoemulsions and is composed of a solid carrier lipid–bioactive ingredient mixture [372].

Protein/peptide nanovehicles may also be used. As an example, curcumin was encapsulated in casein NPs by spray drying their aqueous ethanol solution. This inhibited curcumin

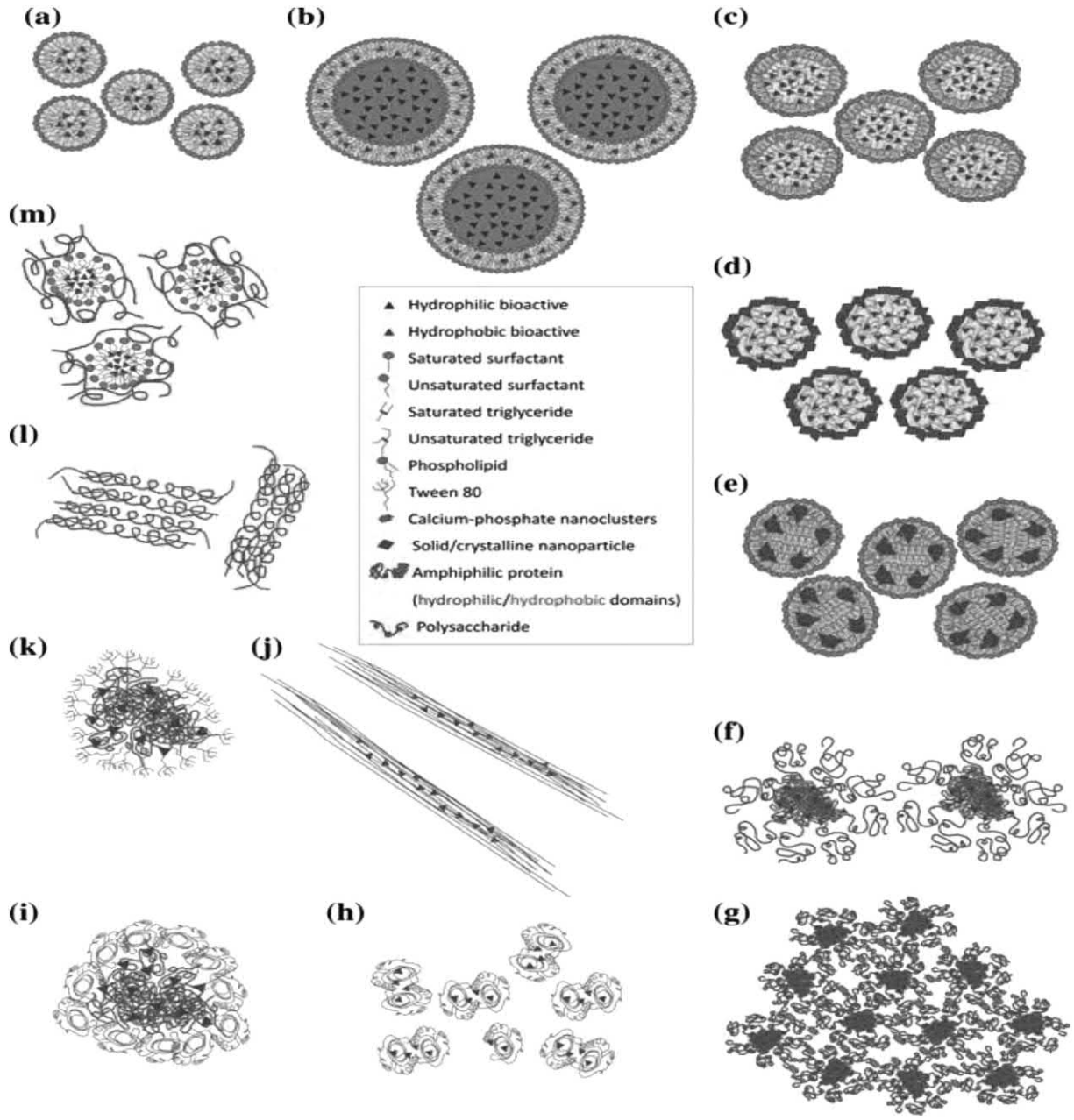

FIGURE 34.27 Types of nanostructured delivery systems recently studied: (a) nanoemulsion, (b) liposomes, (c) nanostructured lipid carriers (NLCs), (d) pickering emulsion, (e) solid–lipid NPs (SLN), (g) reassembled casein micelle, (h) protein-bioactive nanocomplexes, (i) hydrophobic protein, (j) electrospun protein nanofibers, (k) hydrophilic protein, (l) amylose inclusion complexes entrapping fatty acids, (m) polysaccharide-lipid NPs. (From [373].)

crystallinity (Figure 34.27f) [374]. Benzaria et al. [375] reported encapsulation of curcumin in native-like phosphocasein micelles improved by ultrahigh-pressure homogenization (Figure 34.27g). Shpigelman et al. [376] studied nanovehicles for nutraceuticals. They recently formed β-Lg-naringenin nanocomplexes that suppressed naringenin crystallization, in order to promote its bioavailability and increase its effective solubility (Figure 34.27h). Ghasemi and Abbasi [377] encapsulated fish and vegetable oils in casein micelles by use of pH modification.

Chen et al. [378] encapsulated the tangeretin, a hydrophobic flavonoid, in protein. This was achieved by mixing tangeretin and an organic phase containing zein with an aqueous phase containing β-lactoglobulin (Figure 34.27i). Protein–surfactant NPs of Zein–Tween 80 NPs (Figure 34.27k) were proposed as food-grade delivery systems. The NPs had a Tween 80 shell with a thickness around 4 nm and a zein core of 78 nm diameter [379].

The polysaccharide-based NPs could also be prepared by using enzymatically synthesized dextran NPs of 100–450 nm. This was developed in order to entrap hydrophobic nutraceuticals. Generally, dextransucrase generated spherical dextran NPs from sucrose [380]. Mogol et al. [381] used high amylose

corn starch (HACS) for encapsulating omega-3 fatty acids for enriching bread as a functional food as well as for improving thermal stability (Figure 34.27l). Polysaccharide-lipid NPs were prepared for encapsulating quercetin in lecithin/chitosan NPs of about 170 nm size (Figure 34.27 m). The encapsulated product had improved antioxidant properties compared to free quercetin.

34.3.3 Emulsions (Micro- and Nanoemulsions)

Emulsions could be in the form of either water drops in oil or oil drops in water, but in both cases, the droplets must be stabilized to prevent them from recoalescing. Stabilization of the emulsion droplet is usually achieved by the addition of amphiphilic molecules such as emulsifiers that act by decreasing the interfacial tension between the phases as well as increasing the steric hindrances [382]. Food-grade nanoemulsions could be prepared by high-energy methods such as high-pressure homogenization or sonication, or low-energy methods such as maintaining temperature above phase inversion so that nanoemulsions maintain good stability against droplet aggregation [356, 383].

Mehmood [384] developed the delivery of ω-3 fatty acids and vitamin D_3 in cheddar cheese. The study recovery level of vitamin D_3 in cheese was 84%. The fortification of foods by using encapsulated vitamins and other bioactive molecules might have a positive impact on the chemical stability and possibly composition of the resultant products. Vitamin E acetate (tocopheryl acetate) nanoemulsion was produced by using mustard oil and no flocculation was observed during the first 15 days. The nanoemulsion showed improved antimicrobial and antioxidant activity and could potentially be used to increase the shelf life of beverages such as fruit juices [385].

Nanoemulsions of oregano essential oils and clove bud were incorporated into methylcellulose films, and their antimicrobial activity was tested in sliced bread. The study showed that antimicrobial films reduced the counts of molds and yeasts in bread for 15 days [386]. Sari et al. [387] studied both the preparation and physicochemical characterization as well as *in vitro* digestion kinetics of curcumin nanoemulsions. They prepared coarse emulsions by using magnetic stirring at room temperature for different time periods, and fine emulsions were prepared by ultrasound processing of the coarse emulsions. Because curcumin is highly unstable in aqueous food systems, it was encapsulated in a carrier oil as an emulsion form and with an efficiency of 90%; this was found to be more stable than the unencapsulated curcumin for 27 days of storage at 25°C.

34.4 ENCAPSULATED INGREDIENTS AND THEIR APPLICATIONS

Microencapsulation can potentially offer numerous benefits to the materials being encapsulated, particularly in the case of functional food ingredients and nutraceuticals. Various properties of active materials may be changed/modified by encapsulation. For example, handling and flow properties can be improved by converting a liquid to a solid encapsulated form. Hygroscopic materials can be protected from moisture. The stability of functional ingredients that are volatile or sensitive to heat, light, or oxidation can be protected, thereby extending their shelf life. Materials that are otherwise incompatible can be mixed and utilized safely together. Using food fortification as an example, microencapsulation can mask the undesirable taste of some nutrients (i.e., minerals) that may be imparted by the free forms, thus enabling the production of functional foods, say, a nutraceutical beverage, with desirable sensory properties [388]. Currently, several hundred types of microcapsules are being utilized as food additives or as ingredients in functional food formulations throughout North America and elsewhere around the world [24], some of which are described next.

34.4.1 Acidulants

Acidulants are added to foods for a variety of reasons. They can be used as flavor enhancers and modifiers as well as preservation and processing acids. In addition, they facilitate the development of a wide variety of textural effects in foods because of their interaction with other macro- and micromolecules such as proteins, starches, pectins, and gums [389].

Unencapsulated food acids can react with food ingredients to produce many undesirable effects. These include decreased shelf life of citrus-flavored and starch-containing foods (e.g., pudding and pie fillings, in which the acid hydrolyzes the starch), loss of flavor, degradation of color, and separation of ingredients. Encapsulated food acids overcome these and other problems because they preclude oxidation and provide controlled release under specific conditions. Moreover, encapsulated acids reduce hygroscopicity, reduce dusting, and provide a high degree of flowability without clumping.

Encapsulation of acids in a time-release matrix is suggested as a means of avoiding undesirable reactions of acidulants with other food ingredients. The matrix used for forming the encapsulating coat in the acid products is generally a partially hydrogenated vegetable oil, although maltodextrin and emulsifiers are also available for this purpose. The encapsulated acids can be released at the appropriate time in the processing operation either by heating to the melting point of the coating material or by contact with water or a combination of these methods. Several applications of encapsulated acidulants are given next.

34.4.1.1 Meat Processing Aids

In the meat industry, encapsulated acids, such as lactic acid and citric acid, as well as glucono-δ-lactone (GDL) are used to assist in the development of color and flavor in meat emulsions, dry sausage products, restructured meat products, uncooked processed meats and meat-containing products, such as pasta meals. Fat encapsulation allows the acid to survive the blending process, giving a uniform dispersion within the meat formulation. Later, the encapsulated acid controls the drop in pH and prevents the meat from prematurely setting [25]. In a meat preparation, encapsulated acids need to

be released after the later processing stages such as cooking. Early release causes protein binding and the final texture of the product will become brittle and unacceptable.

Cured meat products, especially dry and semidry sausages (e.g., summer sausages, pepperoni, and salami), have historically been prepared using lactic-acid-producing bacterial cultures to develop flavor and lower the pH. Bacteria are added to the meat emulsions and allowed to proliferate until a sufficient amount of lactic acid is generated. Upon its production, the pH drops, binding occurs, and flavor develops. However, such products often tend to have inconsistent flavor, color, and textural characteristics from batch to batch. Uncoated lactic acid and citric acid cannot be added to meat during curing because they react almost instantly with meat proteins, rendering it unsuitable for further processing. Contamination is especially troublesome when the meat processor uses fermented raw meat as the source of bacteria rather than frozen cultures. An encapsulated acid, which is formulated for delayed release under smokehouse temperatures, can be employed as an alternative to the cultures. Acidification by encapsulated acids can improve emulsification and protein binding of emulsified meat and poultry products and impart the "tangy" flavor associated with fermented sausages without the complicated use of lactic acid starter cultures. In effect, encapsulation permits addition of the acidulants prior to stuffing without premature denaturation/binding of meat.

Nearly 50 years ago, encapsulated acids in a heat-rupturable inert vehicle such as ethyl cellulose were developed [390]. The prepared encapsules were mixed with nitrite-treated comminuted meats, and upon thermal processing, the encapsulated acid was released and brought about a lowering of the pH in the meat product and gave rise to rapid development and stabilization of the c.ured meat color. The more acidic conditions within the meat matrix assisted the production of nitrous acid and/or dinitrogen trioxide from the exogenous sodium nitrite. Both nitrous acid and dinitrogen trioxide are potent nitrosating species that interact with the prosthetic heme group of myoglobin, the main haemoprotein pigment of muscle tissue, to form the cooked cured-meat pigment (CCMP), nitorsyl ferrohemochrome.

The effect of encapsulated food acids on restructured pork from prerigor sow meat was studied by Cordray and Huffman [391]. Results from sensory panels indicated that sodium acid pyrophosphate and encapsulated GDL treatments yielded products with a more intense flavor than that of the control sample. Objective analysis revealed no significant ($P > 0.05$) difference in shear value, tensile strength, water-holding capacity, cooked yield, or chilled yield. However, significantly more of the total meat pigment was converted to CCMP in the GLD treatment than that of the control. Lactic acid can also be encapsulated by plating it onto a particle calcium lactate carrier and then encapsulating the carrier and acid with a molten edible lipid [392].

34.4.1.2 Dough Conditioners

The baking industry has long been aware of the need for stable acids and baking soda for use in wet and dry mixes to control the release of carbon dioxide during processing and subsequent baking. Products commonly encapsulated for bakery applications include a variety of leavening system ingredients, as well as vitamin C, acetic acid, lactic acid, potassium sorbate, sorbic acid, calcium propionate, and sodium chloride.

Use of ascorbic acid (vitamin C) for the strengthening and conditioning of bread and roll doughs provides many positive effects to the finished products. Examples of these are stronger sidewalls, uniform crust color, and improved slicing, in addition to a stronger structure that supports the addition of other protein-rich ingredients (such as soybean flour, nonfat milk powder, and wheat germ). However, because ascorbic acid degrades rapidly in the presence of water and oxygen, most of the acid is destroyed before it is needed. Encapsulated in an edible coating, ascorbic acid imparts some of the effect of an oxidizing agent when used alone in natural breads. In combination with bromate, it enables higher amounts of protein-rich ingredients to be utilized without disturbing the grain of the bread to any great extent [393].

For yeast-raised dough, encapsulated salt, potassium sorbate, and sorbic acid are employed because they do not allow the pH to drop too early in the baking process and therefore the yeast can grow. Once baked, however, the mold-inhibiting properties of these ingredients are released into the dough [25].

34.4.1.3 Other Encapsulated Acidulants

Acids are frequently used as liquids but would be easier to handle if they could be supplied in solid forms. Seighman [394] developed a method for encapsulation of food-grade phosphoric acid in a dispersion containing a film-forming agent (hydrogen octenylbutane-dioate-amylodextrin) and a matrix-forming ingredient (modified and hydrolyzed starches). The dispersion is thermally processed and then extruded into a cold aqueous alcohol to solidify the matrix-forming ingredients and to allow the film-forming agent to harden to a vitreous structure.

34.4.2 Flavoring Agents

The development and production of artificial or natural flavors and spices is an ever-expanding field in the food industry. The vast majority of flavor compounds used are a liquid at room temperature and constituents of the flavors tend to show sensitivity toward air, light, irradiation, and elevated temperatures. Moreover, these flavor concentrates are oily and lipophilic materials that can be difficult to work with. Therefore, it is necessary to employ a process to convert these flavor compounds into a more useable form. One of the purposes behind encapsulation in the food industry is the conversion of liquid flavors to dry powders. Microencapsulated flavors provide the convenience of a solid form over a liquid one, with reduced volatility and less oxidation [47, 200, 341]. Microencapsulation has become an attractive option to transform liquid food flavorings into stable and free-flowing powders that are easier to handle and incorporate into a dry food system.

The flavor industry depends heavily on encapsulation as a means of providing solid flavor compounds, which offer them protection until consumption. Flavoring agents and spices are encapsulated by a variety of processes and provide numerous advantages to food processors. Examples of commonly used encapsulated flavors are citrus oils, mint oils, onion and garlic oils, spice oleoresins, and whole spices. Citrus oils are very susceptible to oxidation due to sites of unsaturation in their mono- and sesquiterpenoid structures. Oxidative deterioration results in the development of off-flavors described as painty or turpentine-like. Encapsulated citrus oil, prepared by spray drying in a maltodextrin matrix, has a greater stability than unprotected oil [57].

Because flavors are often volatile materials, the stability of the dry microcapsules is an important consideration. Microcapsules must be stable for an extended period of time. Many volatile liquids can be encapsulated and subsequently dried to form free-flowing powders with minimal loss of activity during storage. Table 34.8 illustrates the stability of encapsulated flavors as a function of storage time in microcapsules of various particle sizes under ambient conditions [40].

Flavors encapsulated by inclusion complexation in β-cyclodextrin were protected against volatilization and attack by oxidation [341, 345]. Storage stability of flavors encapsulated in β-cyclodextrin under "non-stress" conditions at room temperature showed that molecular encapsulation, in most cases, provides an almost perfect preservation of flavors for up to 10 years [345] (Table 34.9).

There has been a great expansion in the development of techniques to encapsulate flavors. A spray dried composition comprising a volatile and/or a liable component in a carrier can be further encapsulated in an extruded glassy matrix. Such a procedure of double encapsulation has recently been developed by Levine et al. [395]. Excellent reviews of microencapsulation technology as it applies to food flavors have previously been written [36, 47, 199, 215, 396–398]. However, it should be noted that details about these techniques are difficult to obtain since they are often trade secrets.

TABLE 34.8
Stability of Microencapsulated Flavors

Encapsulated Flavor	Average Capsule Size (μm)	Storage Period (Days)	Flavor Content in Microcapsules (%) Initial	(%) Final
Cassia	750	730	87.8	86.1
	20	730	63.1	59.2
	600	400	90.2	89.9
	250	500	70.5	76.3
Lemon	40	730	74.0	67.9
	20	730	60.1	59.9
Lime	1000	409	92.5	89.6
Peppermint	50	732	75.3	74.6
	20	730	58.5	56.3

Source: Shahidi and Han [499].

TABLE 34.9
Changes in the Flavor Content of Cyclodextrin–Spice Complexes after 10 years under Normal Storage Conditions

Sample	Flavor content in microcapsules (%) In 1977	In 1987
Garlic oil	10.2–10.4	10.0–10.3
Onion oil	10.4–10.6	10.2–10.4
Caraway oil	10.5	9.9–10.2
Thyme oil	70.5	9.0–9.2
Lemon oil	74.0	8.6–8.8
Anise oil	60.1	9.0–9.3
Peppermint	92.5	9.0–9.2
Marjoram	75.3	8.0–8.2
Orange	58.5	6.0–7.0
Tarragon	10.0–10.3	8.8–9.0
Mustard	10.8–11.0	11.0–11.2

Source: Shahidi and Han [499].

34.4.3 Sweeteners

Sweeteners are often subjected to the effects of moisture and/or temperature. Encapsulation of sweeteners, namely, sugars and other nutritive or artificial sweeteners, reduces their hygroscopicity, improves their flowability, and prolongs their sweetness perception. Sugar that has been encapsulated with fat and incorporated into chewing gum requires more shear and higher temperatures to release its sweetness than uncoated sugar, which dissolves more rapidly in the mouth.

Patents awarded to the encapsulation of sweeteners emerged mainly in the 1980s, as the technical development of encapsulation allowed their commercial manufacture. Among these, aspartame is the most widely studied sweetener. Aspartame is the methyl ester of a dipeptide made from two amino acids, phenylalanine and aspartic acid (aspartate). Although this white, odorless, and crystalline powder has a very intense sweetness, approximately 180 to 220 times sweeter than sucrose, the potential for its use in food has, in the past, been limited. At high temperatures, aspartame degrades into its amino acids, aspartic acid and phenylalanine, which is accompanied by a loss of sweetness. This internationally marketed sweetener has now been encapsulated by many methods.

Patents awarded to Cea et al. [399, 400] are mainly on the encapsulation of APM (L-aspartyl-L-phenylalanine methyl ester) as a chewing gum composition. It has been claimed that the encapsulated APM overcomes difficulties experienced in the use of APM with respect to its stability in the presence of water or elevated temperature [399, 400]. Yang and coworkers

developed a process for encapsulating aspartame in a film composed of high molecular weight polyvinyl acetate and a hydrophobic plasticizer (mono- or diacylglycerol with fatty acid chains of 16–22 carbon atoms) [401–403]. In this process, active ingredients, including soluble dietary fibers, flavoring agents, and drugs, can also be encapsulated. The product can be used to give chewing gum an extended shelf life, with highly controlled release of active ingredients [401].

A process developed by Cherukuri and coworkers can be used to produce a stable delivery system. It comprises a dipeptide or amino acid sweetener or flavorant or mixture thereof encapsulated in a mixture of fat and high-melting-point polyethylene wax [404–406]. Gas chromatographic analyses were used to measure the retention of orange, synthetic peppermint, and natural lemon flavors that had been cocrystallized and then stored in polyethylene bags under ambient conditions. Data indicated that there was no significant change in flavor retention for up to 15 weeks of storage. Results from oxidation studies [407] showed that peanut-butter-flavored products had a very good shelf life, even after storage for an appreciable period of time. Chen et al. [80, 81, 407, 408] have published a number of patents in this area. Some typical examples of products encapsulated by cocrystallization are listed in Table 34.10.

34.4.4 Colorants

Natural colors such as annatto, β-carotene, and turmeric present solubility problems during their use and may create dust clouds. Encapsulated colors are easier to handle and offer improved solubility, stability to oxidation, and control over stratification from dry blends. Synthetic colors, together with other food ingredients, can also be encapsulated for improving their stabilities [409].

A technique for solubilizing oily substances in micellar solutions of protein and carbohydrates was applied by Ono [167] in order to achieve encapsulation of two oil-soluble pigments, namely, paprika oleoresin and β-carotene. The pigment in oil was solubilized in an aqueous solution containing 60% (w/w) corn syrup solids and 1% (w/w) polypeptone. The solubilized mixture so obtained was solidified by vacuum drying at 60°C and formed into granules by crusting and sieving. These granules containing approximately 12% pigment-containing oil underwent virtually no discoloration during storage for 20 days at 60°C or when subjected to irradiation under a fluorescent lamp. Dispersibility of the pigments in water was improved by their encapsulation in a protein–carbohydrate matrix [167].

Ciliberto and Kramer [410] developed an encapsulation process for producing granular water-soluble food ingredients that otherwise deteriorated on exposure to the atmosphere (such as coloring agent). It was claimed that the resulting coated particles had a long shelf-life and were still substantially instantaneously soluble in water. Studies on encapsulation of preformed cooked cured-meat pigment (CCMP) showed that the CCMP may be stabilized effectively by its encapsulation in food-grade starch-based wall materials. The color stability of the treated meat products was found similar to their nitrite-cured analog [411].

TABLE 34.10
Examples of Products Encapsulated by Cocrystallization

Flavored sugar crystals	Brown sugar, chocolate, honey, molasses, and peanut butter granules
Fruit juice crystals	Cranberry, grape, orange, raspberry, and strawberry juices
Essential oil powders	Cinnamon, lemon, orange, and peppermint oils
Dry flavors	Barbecue, beef fat, butterscotch, chocolate, maple, and smoke flavors
Volatile substances	Acetaldehyde and diacetyl

34.4.5 Lipids

Lipids contribute to more than 30% of the dietary energy of North Americans, and similar figures apply to many other affluent societies. Use of lipids/fats are commonplace in food processing practices, but the susceptibility of lipids to oxidative degradation during processing and storage is always a concern; particular attention must be paid to foodstuffs containing higher proportions of polyunsaturated fatty acids (PUFA). One possible way to protect lipids against oxidative deterioration is via encapsulation. Early research in this area was mainly focused on production of encapsulated lipids for animal feed [412–415], but more recently, encapsulated high-fat powders or shortenings have become available in human food formulations [416].

Because of the pro-health benefits of fish oils, encapsulated oils have been available in health food stores, pharmacies, and supermarkets for a number of years. These fish oils contain long-chain omega-3 PUFA, such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA), whose beneficial effects have been ascribed to their ability to lower blood serum triacylglycerol and cholesterol levels [417, 418]. Whereas DHA is essential for proper functioning of the eye and may have a structural role in the brain, EPA serves as a precursor to eicosanoid compounds [419] and has therapeutic benefits in human cardiovascular diseases [141, 420, 421]. It should be noted that fish oils are exceptionally susceptible to autoxidation and can form complex mixtures of high molecular weight oxidation products. Shukla and Perkins [422] reported that because of the unknown health effects of the oxidative polymeric materials and their high level in some encapsulated oils, caution should be exercised when ingesting fish oil capsules on a regular basis. However, encapsulation can enhance the oxidative stability of these oils.

Gejl-Hansen and Flink [423] freeze dried an aqueous emulsion of linoleic acid in a maltodextrin coating in the presence of detergents. The microencapsulated linoleic acid was not susceptible to oxidative deterioration even though more effective encapsulating wall materials could have been used. Ono and Aoyama [168] reported that vacuum dried rice brain oil embedded in granules containing corn syrup solids and

pork polypeptone did not undergo much oxidation upon exposure to air at a high temperature for a few weeks. Taguchi et al. [424] reported the oxidative stability of sardine oil embedded in spray dried egg white powder and use of the product as a source of omega-3 PUFA for fortification of cookies. These authors reported that use of microencapsulated sardine oil in fortified cookies did not affect their sensory quality.

The antioxidative effects of spray dried powders, at various water activities, prepared from alcoholic solutions of gliadin, linoleic acid, and palmitic acid, were compared against powders prepared by simple mixing of these components in the same portions and against gelatine or starch powders substituted for gliadin by Iwami et al. [425]. It is reported that the microcapsules obtained from the experiment were highly resistant to oxidative deterioration during long-term storage at various A_w's [425]. Shahidi and Wanasundara [426] spray dried an emulsion of seal blubber oil, which contained 21–26% long-chain omega-3 PUFA, with either β-cyclodextrin, corn syrup solids, or maltodextrins. They found that β-cyclodextrin was the most effective entrapping agent and prevented oxidative deterioration of seal blubber oil.

34.4.6 Vitamins and Minerals

Most vitamins cannot be synthesized by the body and must be supplied by the diet [427]. Because vitamins are such important nutritional and dietary factors, processed foods are often enriched or fortified with vitamins. Table 34.11 presents the recommended daily allowances for vitamins A, D, E, K, C, B_6, B_{12}, folic acid, thiamine, riboflavin, and niacin as compiled by the National Academy of Sciences' Food Nutrition Board [428]. Vitamins and minerals are often added to dry mixes to fortify a variety of foods.

Encapsulation of vitamins and minerals offers many advantages as it reduces off-flavors contributed by certain vitamins and minerals, permits time release of the nutrients, enhances stability of vitamins to extremes in temperature and moisture, and reduces each nutrient's reaction with other ingredients. Encapsulation also improves flow properties and reduces dusting when nutrients are added to dry mixes. Both fat- and water-soluble vitamins may be encapsulated with a variety of coatings to provide many advantages. Hall and Pondell [429] developed a process to encapsulate vitamin or mineral particles. The coating matrix for this process was chiefly ethyl cellulose together with propylene glycol monoester and acetylated monoglycerol. Vitamins and minerals can also be encapsulated in fat [430] or in starch matrices [431].

For encapsulation of water-soluble vitamins, ethylcellulose is useful because it is water-insoluble, and coatings with increased thickness reduce the water permeability of the prepared capsules. Thiamine enrichment of some bakery products such as devil's food cake, ginger snaps, and soda crackers, has always been unsuccessful due to the vitamin destruction in the neutral or alkaline pH. A procedure for microencapsulating thiamine in an ethylcellulose coating to protect it from alkaline conditions experienced in bakery products, and to mask its undesirable bitter taste, has been developed [432].

Riboflavin, thiamine, and niacin are partially destroyed during processing and cooking of pasta products. Studies on unprotected versus encapsulated thiamine, riboflavin, and niacin in cooked enriched spaghetti showed that concentrations of the three B vitamins tested were higher in cooked pasta that contained encapsulated vitamins [433].

Lipid-soluble vitamins lose their activity due to isomerism, anhydrovitamin formation, oxidation, and photochemical reactions [341]. Losses of vitamins in fortified foods can be minimized if they are added as cyclodextrin complexes [341] or gelatine-encapsulated beadlets [434]. It was found that the stability of vitamin A in skim milk was substantially increased by encapsulation in gelatine. Loss of the vitamin in fortified milk powder was minimal even when heated at 100°C for 9 min or stored at 28°C for 40 weeks [434]. Table 34.12 presents the stability data of vitamin A palmitate, of 325,000 units per gram potency, encapsulated in a modified gelatine film [40]. The data indicate that the rate of vitamin A degradation under the test conditions is significantly reduced by microencapsulation.

TABLE 34.11
Recommended Dietary Allowances

Vitamin	Men	Women	Children to Age 11
Fat-soluble			
Vitamin A (retinol, μg)	1000	800	400–700
Vitamin D (cholecalciferol, μg)	5–10	5–10	10
Vitamin E (α-tocopherol, mg)	10	8	6–7
Vitamin K (μg)	45–80	45–65	15–30
Water-soluble			
Vitamin C (mg)	60	60	40–45
Vitamin B_1 (thiamine, mg)	1.5	1.1	0.7–1.0
Vitamin B_2 (riboflavin, mg)	1.7	1.3	0.8–1.2
Niacin (mg)	19	15	9–13
Vitamin B_6 (pyridoxine, mg)	2.0	1.6	1.0–1.4
Vitamin B_{12} (μg)	2.0	2.0	0.7–1.4
Folic acid (μg)	200	180	50–100

TABLE 34.12
Stability of Vitamin A Palmitate at 45°C and 75% Relative Humidity

	Percentage of Potency Retained	
Time (Days)	Raw Oil	Microencapsulated
5	86.1	98.3
15	84.2	97.8
42	76.2	94.2
56	69.9	94.1

Source: Shahidi and Han [499].

A well-designed phase-separation technique for encapsulation of vitamin A has been developed by Markus and Peleh [435]. The matrix components used consisted of substituted cellulosic materials, fatty acids, or a variety of proteins. Antioxidants such as butylated hydroxytoluene (BHT) and ethoxyquin were incorporated in the formulations. It has been claimed that the capsules prepared with substituted cellulosic materials gave the best protection to vitamin A from degradation [435].

Iron compounds have been encapsulated to improve the color, odor, and shelf life of fortified products. Encapsulation reduced the ability of iron to react with other food ingredients and also lightened the color of an unspecified type of electrolytic iron [436]. The process for encapsulation of ferrous sulfate was developed by Jackel and Belshaw [437] in the 1970s. It is reported that the encapsulated $FeSO_4$, a fine, white, free-flowing powder, can withstand 6 months of storage without any deteriorative change. Harrison et al. [438] examined the effect of iron in various forms on the oxidation of lipids in white flour. When subjected to an accelerated stability test (stored at 50°C), flours enriched with ferrous sulfate, fat enriched with ferrous sulfate, electrolytic iron powder, and carbonyl iron powder, developed an unacceptable oxidized flavor after 8 days. However, oxidation was not detected in flour stored at room temperature for 2 years [438].

Soy milk beverages have gained attention as possible alternatives to cow's milk. However, soy milk is nutritionally inferior to cow's milk with respect to its calcium content. Attempts to fortify soy milk with calcium have been unsuccessful since soy protein is coagulated and precipitated by calcium [439, 440]. Hirotsuka et al. [440] found that calcium coated with lecithin to form liposomes could be added to soy milk without undesirable calcium–protein interactions. The technology was successful in fortifying 100 g of soy milk with an additional 120 mg of calcium.

34.4.7 Enzymes

Enzymes are being used increasingly in the food industry for a wide variety of applications. Encapsulation of enzymes could enhance their properties in different ways. The first and foremost of these concerns stability. The complex biochemical structure of the enzyme can make it highly vulnerable to inactivation by other components or conditions within the food system. By segregating it inside a microcapsule, it can be maintained in conditions that could otherwise be very harmful to it. A variety of other stabilizing materials can be encapsulated alongside the enzyme to protect them from different antagonistic effects. Inhibitory agents and harmful ions will be excluded from the capsule. Penetrating ions can be removed by buffers or chelating agents, and oxidative damage may be prevented by the use of antioxidants. Thermostabilizers such as sugars will protect against extreme processing conditions such as dehydration or freezing. Further stabilization may be achieved by simply maintaining the enzyme in a concentrated form rather than allowing it to become diluted into the bulk-food phase.

As long as it remains encapsulated, the enzyme will be isolated from its substrate and therefore latent and passive within the food matrix. By selecting a capsule with appropriate properties, we can choose when, where, and how to interact it with its intended substrate. By altering the surface properties of the microcapsules, they can often be made to accumulate at a particular microscopic location within the food. When they eventually break down, the enzyme activity will be concentrated at the intended target site rather than nonspecifically dispersed throughout the food. In this way, enzymes can be used much more selectively and with far greater efficiency than their normal usage would allow.

The timing of enzyme release can be controlled by selecting a microcapsule according to its stability properties within a particular food system. A low stability will lead to early release in the food process, whereas a more stable one will allow postponement of its release. This is very useful when early release is undesirable and enzyme action is not needed until a later step of a multistage process.

Considerable progress in research for the control of cheese ripening using encapsulated enzymes has been achieved [441–449]. The principles involved in this application provide a good illustration of how encapsulation can be generally applied in the food industry, as it has been reviewed by Kirby and Law [450]. Other enzymes such as lipase [451, 452] and invertase [453, 454] have also been encapsulated for applications in food processing.

34.4.8 Microorganisms

Encapsulation of viable bacterial cells has several advantages over encapsulation of isolated enzymes such as those in cheese ripening. The stability of enzymes in intact cells is greater than in extracts. Production achieved by cells is also easily manipulated by controlling substrate concentration in the microcapsules [448].

Kim and Olson [449] reported that cells of *Brevibacterium linens* were successfully entrapped in milk-fat-coated microcapsules. It is believed that the bacteria using methionine to produce methanethiol and other sulfur compounds make a major contribution to the cheddar cheese flavor of low-fat cheese products. Microencapsulated microorganisms may be helpful in reducing the ripening time of blue cheese or in imparting blue cheese flavor to other foods. Spores of *Penicillium roqueforti* were encapsulated in a milk fat coating matrix [455]. The microenvironment provided by the microcapsules enhanced methyl ketone production by spore enzymes.

Recently, intensive research efforts have been focused on protecting the viability of bacterial cultures, which demonstrate probiotic activity, during a product's manufacture, storage, and gastric transit. Studies have demonstrated that probiotic cultures can be significantly protected via encapsulation using a variety of carriers. In essence, microencapsulation has been at the forefront of the functional foods and nutraceuticals revolution, as it relates to probiotics.

Probiotics are described as "live microorganisms which when administered in adequate numbers confer a health benefit to the host" [456]. These supplements contain beneficial,

friendly bacteria of the *Lactobacillus* and *Bifidobacterium* species, and are formulated to reflect the composition of healthy gut flora; they have a direct impact on both the host's metabolism and physiology. In order for a microorganism to be classified as a probiotic, it must be (i) isolated from humans, (ii) compatible with humans, (iii) safe for humans, (iv) able to survive the acid conditions in the stomach, (v) able to grow and colonize in the gut, (vi) antagonistic to pathogenic bacteria, and (vii) have scientifically proven efficacy. Moreover, probiotics share a number of common traits such as a generally regarded as safe (GRAS) status, acid and bile tolerance, and an ability to adhere to intestinal cells [457]. Though probiotics are commonly added to fermented milks, yogurts, and cheeses (i.e., as functional foods), they are also available as dietary supplements, where the probiotic is in the form of a dried encapsulated product or capsule [458]. Clinical studies have shown beneficial health effects from the consumption of probiotics of the *Lactobacillus* and *Bifidobacterium* species, and these include reduction of rotavirus diarrhea, alleviation of lactose-intolerance symptoms, reduction of harmful intestinal microbial enzyme activity, decrease in fecal mutagenicity, aid in digestion, prevention of illness by inhibiting the growth of pathogenic and putrefactive bacteria in the gastrointestinal (GI) tract, and possibly assistance in the reduction of cholesterol [459–462].

In Japan, there is a long tradition of believing that health is dependent on food and in particular that the maintenance of a population of beneficial bacteria is important. The addition of probiotics to foods is seen as an effective means of restoring some of the initial beneficial food-associated microflora that may be destroyed during microbial reduction treatments such as pasteurization. The Japanese have long believed that probiotic bacteria must arrive at the site of action in large enough numbers to exert their effect and this might most easily be achieved by using resistant bacteria or by providing large numbers of live bacteria when consumed to compensate for losses during passage through the upper GI tract. The Fermented Milks and Lactic Acid Bacteria Beverages Association in Japan has set a minimum of 10^7 bifidobacteria or CFU/mL of product [463, 464]. Furthermore, the probiotic food product must be consumed regularly to ensure that a sufficient "dose" of live bacteria is delivered to the gut. Consequently, technological issues relating to the development of foods containing these bacteria in sufficient numbers throughout shelf life need to be overcome as well as a means of stabilization following ingestion, that is, during exposure to the adverse conditions of the human GI tract [458]. This is where encapsulation technology has stepped in to give a helping hand. The probiotic food industry has been increasingly utilizing encapsulation as a means of protecting live cells from extremes of heat or moisture, such as those experienced during drying and storage [465, 466].

Spray dried powders possessing high numbers of viable probiotics is a convenient means of storage and transport of probiotic cultures and their subsequent application to functional foods. Most probiotic lactobacilli, however, do not survive well during the temperature and osmotic stresses they face when spray dried [467, 468]. Thermal and dehydration damage to cell membranes and proteins leads to bacterial cell injury and eventually death. To overcome this problem one approach has been the addition of thermoprotectants to the media prior to drying. Desmond et al. [469] reported that the incorporation of soluble fiber (as a prebiotic, an energy source for the probiotic) and gum acacia to a milk-based medium prior to spray drying of the probiotic, *Lactobacillus paracasei* NFBC 338, increased the culture's viability during drying and powder storage compared with milk powder alone. In this situation the encapsulated product not only had better probiotic viability, but it can also be regarded as a synbiotic given the presence of both probiotics and prebiotics. Other investigated prebiotics such as inulin and polydextrose did not enhance probiotic viability during drying or powder storage [470].

Several methods of microencapsulation of probiotic bacteria have been reported; these include spray drying, extrusion, emulsion, and phase separation [10, 471]. In a study by Guerin et al. [472] *Bifidobacterium bifidum* cells encapsulated in gel beads composed of alginate, pectin, and whey proteins, and surrounded by two membranes exhibited good survival at pH 2.5 for up to 2 h, while free cells did not survive under these conditions. Furthermore, protection was afforded by this system when the cells were exposed to bile salt solutions. Selmer-Olsen et al. [473] reported that encapsulated lactobacilli in calcium-alginate beads had improved heat tolerance, and O'Riordan et al. [466] demonstrated that this technology also prolonged the viability of spray dried *Bifidobacterium ruminatium* during storage. Probiotic bacteria have also been microencapsulated in a matrix of vegetable fatty acids. This technique broadens the range of ingredients with which probiotics can be blended and formulated. Moreover, the passage through the GI tract is very stressful for probiotics, especially on an empty stomach where the pH can be as low as 1.5; thus, the hydrophobic coating surrounding the microencapsulated bacteria protects the fragile microbial cells and allows them to pass into the intestine. According to Frost and Sullivan [4] the future for probiotics is bright because their encapsulation in hydrocolloid beads is helping to improve their survival rate right through both processing and digestion. As the technology matures and improves, it is hoped in time that a mechanism for targeting the release of beneficial bacteria in specific sites of the human GI tract will be provided.

34.4.9 Gases

Some hard candies can be made with entrapped carbon dioxide gas [451]. Confections made with encapsulated carbon dioxide produce a sizzling effect on the tongue as the candy melts in the mouth. The candy is produced by incorporating gas at a pressure of 50 to 1000 psi into the molten sugar. Concentrations of carbon dioxide in the candy range from 0.5 to 15 ml per g sugar [451]. Gas can also be injected into the encapsulation system and be coated together with the foaming and aromatic core mixtures [474].

34.4.10 Other Food Additives

Almost all food additives can theoretically and technically be encapsulated. However, only some encapsulated additives

are commercially available since there are many factors that have to be taken into consideration before the process leads to commercial manufacture. Research has been done to encapsulate food preservatives such as monocapric acid [475] and oleic acid [476]. The process for preparing a coated-particle salt substitute composition was described by Meyer [477]. Other studies suggested that encapsulated antioxidants could be beneficial to food preservation [478]. It could be expected that there are many new encapsulated food ingredients that will be produced, and these could contribute greatly to further development of food processing and preservation.

34.5 CONTROLLED-RELEASE MECHANISMS AND THEIR EFFECTS

Encapsulation allows reactive ingredients to be separated from the environment until their release is desired. Though separation is the goal of encapsulation, release mechanisms of the core material must also be considered. In fact, when designing a custom encapsulated food ingredient, one must determine the desired release mechanism and a method for ensuring quality control. For example, in home-baked pizza products, sodium bicarbonate can be encapsulated to prevent early release of the bicarbonate and delay the reaction of the leavening phosphate until the crust reaches a particular temperature in the oven. Controlled release technology can help a wide variety of nutritional supplements deliver their payloads more effectively while heightening produce resale frequency for brands and retailers. In essence, a well-controlled release of core material is a very important property of microcapsules. For example, a substance in formulated food may be released upon consumption but prevented from diffusing throughout the product during processing operations (e.g., flavors, nutrients). Similarly, an additive may be released in a specific processing step, but protected in preceding operations (e.g., acids, leavening agents, cross-linking agents) [479].

Because the physical and chemical properties of volatile compounds are governed by their structures and cannot be changed, one has to manipulate the choice of the encapsulation matrix as well as the formulation of the flavor itself if the flavor is a compound flavor. By picking a capsule matrix with limited selectivity, which may in fact be chosen to discriminate against vapor pressure differences and the desired flux rate (to release slowly or quickly but uniformly), flavor imbalances can be minimized. Additionally, if the flavor is a formulated one, there may be some opportunity to choose flavor compounds that will have similar release rates. Such well-controlled release delivery systems present the food technologist with exciting opportunities for improving the performance of existing food processes, as well as for the development of entirely new ones [479, 480]. However, in order to address the issue of controlled release, one needs to examine the basic principles of controlling the release of encapsulated materials and then consider which technologies can be applied in the food industry. The various mechanisms of release from controlled-release delivery systems in consumer products are provided in Table 34.13 [481].

TABLE 34.13
Mechanisms of Release from Controlled-Release Delivery Systems in Consumer Products

Diffusion-controlled release	Membrane-controlled release
Pressure-activated release	Tearing or peeling release
Solvent-activated release	Osmotic-controlled release
pH-sensitive release	Temperature-sensitive release
Melting-activated release	Hybrid release

Source: Brannon-Peppas [480].

34.5.1 Release Rate

Release rates that are achievable from a single microcapsule are generally zero, half, or first order. Zero-order release occurs when the core is a pure material that may be released through the wall of a microcapsule as a pure material. Half-order release generally occurs with matrix particles, whereas first-order release occurs when the core material is actually a solution trapped within a solid matrix [479]. As the solute material releases from the capsule, a desired concentration of solute is reached.

A mixture of microcapsules will include a distribution of capsules varying in size and wall thickness. The effect, therefore, is to produce a release rate different from zero-, half-, or first-order because of the ensemble of microcapsules. Thus, it is desirable to carefully examine the experimental basis of the release rate from an ensemble of microcapsules, and to recognize the deviation from theory due to the distribution in size and wall thickness [24]. Numerous factors affecting the release rate of core materials are summarized in Table 34.14.

34.5.2 Release Mechanisms

The coating not only protects the core material from moisture, light, oxygen, other food ingredients, and additional external agents [307], but it allows/assists in controlling the release of core materials. Thus, the release of the core material is dependent upon the type and geometry of the particle and the wall material used to form the microcapsule. These factors dictate the mechanism of release for the capsule, which may be based on solvent effects, diffusion, degradation, or particle fracture

TABLE 34.14
Parameters Affecting the Release Rate of Core Materials

Coating properties	Density, crystallinity, orientation, solubility, plasticizer level, cross-linking, pretreatments
Capsule properties	Size, wall thickness, configuration, conformity, coating layers, posttreatment
Experimental parameters	Temperature, pH, moisture, solvent, mechanical action, partial pressure differential (inside and outside coating)

Source: Shahidi and Han [499].

[482]. A variety of release mechanisms, which have been proposed for microcapsules, are summarized next.

34.5.2.1 Fracturation or Pressure-Activated Release

A number of controlled-release systems prepared primarily by coacervation technology depend on pressure for release of the active core [483]. The coating can be fractured or broken open by external forces, such as pressure, shearing, ultrasonics, or by internal forces, as would occur in a microcapsule having a permeation-selective coating. Both fracturation and diffusion involve the controlled release of volatile materials; however, a slow release of core material from the capsule in the case of fracturation is a detriment rather than an attribute. A completely impermeable capsule is needed that releases only on rupture. For example, capsules made from hardened fats or waxes are insoluble in water but can be made to release their contents by mechanical breakage, e.g., shear, or by increasing the temperature to the melting point of the fat (see Section 34.5.2.4). The act of chewing is the most commonly used mechanical release means. It is also possible to get release of the core substance by incorporation of a swelling agent into the core substance or by an electromagnetic method using discharge or magnetic force. The force-fractured release is accomplished in a relatively shorter duration beginning at certain controlled conditions compared to the other release mechanisms.

34.5.2.2 Diffusion

The mechanism involved in diffusion is to limit the release of core material from within the capsule to the surface of the particle by controlling the rate of diffusion of the active compound. The bulk of the capsule material itself may control the release (i.e., matrix-controlled release) or a membrane may be added to the capsule for doing so (i.e., membrane-controlled release). Most microcapsules have thin walls that may function as a semipermeable membrane. Furthermore, because microcapsules are very small in size, they have a very large surface area per unit weight. Hence, controlled release is frequently accomplished through a diffusion-controlled process [484].

Diffusion release depends upon the kinetic relationship between the core and wall materials and the rate at which the core material is able to pass through the outer wall. It is strictly governed by the chemical properties of the microcapsule and by the physical properties of the wall material such as the matrix structure and pore sizes [482]. Diffusion is a permeation process driven by a concentration gradient or interchain attractive forces [485]. In other words, it is controlled by the solubility of a component in the matrix (this establishes a concentration gradient in the matrix for driving diffusion) and the permeability of the component through the matrix. In the absence of cracks, pinholes, or other flaws, the primary mechanism for core materials to flow through a wall or coating is activated by diffusion, i.e., the penetrant dissolves in the film matrix at the high concentration side, diffuses through the film driven by a concentration gradient (i.e., Fick's law, $I_A = -D_{AB}\, dC_A/dy$, where I_A is the flux of the core material in the y direction, D_{AB} is the diffusivity, and dC_A/dy is the concentration gradient), and evaporates from the other surface. It should be noted that if the food components were not soluble in the matrix, it would not enter the matrix to diffuse through, irrespective of the matrix's pore size.

Diffusion also depends upon the size, shape, vapor pressure, and polarity of the penetrating molecules as well as the segmental motion of polymer chains [485, 486]. This also includes interchain attractive forces such as hydrogen bonding and van der Waals' interactions, degree of cross-linking, and the amount of crystallinity [487]. In general, cross-linking of a matrix has little meaning in most food applications. This is because very few situations exist where the matrix can be cross-linked considering the limitations imposed by having food-approved materials [484]. However, cross-linking of proteins as a consequence of Maillard reactions can occur and possibly influence the diffusion of solutes in heated protein-based encapsulation matrices (e.g., gelatine). Thus, the greater the degree of cross-linking, the lesser the rate of diffusion through the matrix (hence, a readily controllable process of making a controlled-release capsule).

The problem of uniform releasing of the aroma of an encapsulated flavor into food should be noted. Because a flavor consists of aroma compounds with a range of volatility, their release, for example, into the headspace of a food package will not be uniform and therefore a balanced characteristic food aroma may not be achieved [488]. The volatility or vapor pressure of these different compounds and their resistances to diffusion will affect their rate. Thus, aromas could become imbalanced as the constituents diffuse through the capsule.

For most physical methods, it is known that the success of encapsulation depends on the formation of a metastable amorphous structure, a glass, with a very low permeability to the organic compounds that are encapsulated within it. In drying processes, the presence of sugar and/or polymers in the encapsulation system reduces the water content. Reducing the water content lowers the glass transition temperature and the resulting amorphous matrix is impermeable to organic compounds as well as to oxygen. However, permeability to water remains finite. This phenomenon, also known as the selective diffusion theory of Thijssen and Rulken [489], is the basis for encapsulation using spray drying and freeze drying [479]. In spray drying, upon droplet formation, rapid evaporation from the surface produces a surface layer in which the selective diffusion mechanism operates. In freeze drying, upon water crystallization, the nonfrozen solution is viscous, and the diffusion of core materials is retarded. At the beginning of freeze drying, the surface of this solution becomes an amorphous solid in which selective diffusion comes into play.

The permeability of the coating structure can be changed by controlled conditions. The physical state of the food polymer has a considerable role in influencing diffusion and thus release of the core material. The physicochemical principles governing the softening or glass transition of the encapsulating materials have been studied by several researchers [490–493]. These investigations have shown that the release occurs when the glassy, impermeable structure undergoes a transition to a more mobile rubbery state (Figure 34.28). Thus, the

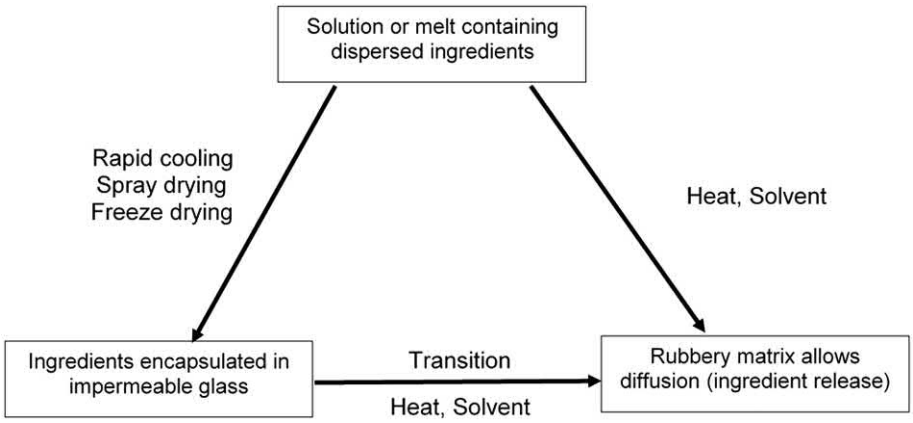

FIGURE 34.28 Preparation and release of core ingredients from microcapsules. (From Karel and Langer [478].)

glass–rubber transition of a matrix material is a relevant consideration when evaluating release properties. The relation of transition temperature to the composition of encapsulating formulations has been studied by To and Flink [494] and Levine and Slade [491] for the case of starch-derived encapsulating agents. It must be noted, however, that even after the critical moisture content or the critical temperature is exceeded, the rate of release is also a function of water content, temperature, and time [495]. This fact allows the generation of controlled-release systems. The maltodextrins and similar materials with controlled collapse temperatures are important not only as encapsulating agents but are also extremely useful in protecting enzymes and other sensitive biological materials during dehydration and subsequent storage. The principles are similar in that the sensitive materials are placed in a medium in which their mobility is restricted.

34.5.2.3 Solvent-Activated Release

Solvent-activated release is the most common controlled-release mechanism used in the food industry. Since most encapsulating matrices are water-soluble, the water in the food product dissolves away the microcapsule thereby liberating its content to the food, or it causes the capsule to swell to either begin or enhance the release of the core material. However, water-insoluble coatings can also be dissolved by selecting an appropriate solvent. Encapsulated agents are often added to dry food products such as dry beverages, and cake and soup mixes. The encapsulated flavors in these products are released upon rehydration [484]. Their release may be a sudden burst, or a continued or delayed delivery regulated by controlling the rate of wall solubility, the swelling of the wall material, pH effects, or changes in the ionic strength of the surrounding medium [482].

Although most traditional wall materials will rapidly release the core material once they are rehydrated, microcapsule matrices may be modified to release the active material at a desired point in time. Osmotic-controlled release is similar to solvent-activated release in that the core of the particle adsorbs a solvent (usually water) over time and swells until the capsule bursts [481]. For any food ingredient that is first encapsulated in a hydrophilic matrix and then coated with a lipophilic once, osmotic-controlled release functions to a limited extent. The encapsulated product will eventually swell and either expand the surface coating causing cracks or fractures, or rupture entirely.

34.5.2.4 Melting-Activated Release

The integrity of the coating can be destroyed by thermal means. This mechanism of release involves the melting of the capsule wall (or a protective coating that has been placed on the capsule wall) to release the active material. Because there are numerous meltable materials that are approved for food use (e.g., lipids, waxes, and modified lipids), this method of release is easily accomplished, but its applications are limited. In general, salts, nutrients, leavening agents, and some water-soluble flavoring agents have been protected by hydrophobic coatings to curtail release of the active ingredient into the food until the baking process. The hydrophobic coating and core material must be immiscible with one another in order to avoid migration of the active ingredient through the wall material. This limits the usefulness of the technique for many flavor applications. On the other hand, an already encapsulated flavor prepared by spray drying can be coated with a hydrophobic matrix via centrifugal coating or the fluidized bed technique. In this manner, the secondary coating on the flavor provides melt release properties [496]. The major problem in this approach, however, is the dilution of the flavoring by additional wall material and the extra cost involved.

34.5.2.5 Biodegradation and pH-Sensitive Release

Release from microcapsules can be accomplished by biodegradation processes if the coatings lend themselves to such degradative mechanisms. Lipid coatings may be degraded by the action of lipases [497]. Karel and Langer [479] released enzymes from liposomes using pH as a stimulant to initiate release. They postulated that pH changes destabilized the phospholipid-based liposomal structure, thereby releasing the enzymes from the liposome core.

34.6 CONCLUSION

Encapsulation is an effective preservation method for different food components as well as for bioactive compounds, including

antioxidants, lipids, peptides, vitamins, minerals, fatty acids, and probiotics. The main goal consideration should be to choose the appropriate encapsulation technique as well as the proper encapsulating material. Microencapsulation and nanoencapsulation have shown greater potential in improving the effectiveness of the delivery of bioactive compounds, which contribute to improved health. Different techniques of encapsulation are still emerging with their own advantages and disadvantages. Techniques such as spray drying, electrohydrodynamic techniques, spray cooling and spray chilling, freeze drying, fluidized bed coating, extrusion, coacervation, liposome, and emulsification are useful techniques for encapsulation of food ingredients. Furthermore, freeze drying and extrusion remain unique techniques for encapsulation of heat-sensitive ingredients. However, all encapsulation techniques ultimately depend on how to produce encapsulates in the powder form. Currently, freeze drying and spray drying are widely used drying techniques in the encapsulation process. Nevertheless, freeze drying is still 4–7 times more expensive than spray drying. Each encapsulation technique has several unique operating variables and factors that affect the quality of the final encapsulates; these factors need to be considered and carefully optimized. However, possible risks of encapsulation materials to human health are still unknown. Thus, regulatory national bodies may need to move ahead with initiatives to control and monitor proper development of encapsulated food products and ingredients.

34.7 FUTURE DIRECTIONS

Many encapsulation techniques can be developed with high efficacy. Further studies that investigate techniques other than microencapsulation, nanoencapsulation, and emulsions with different combinations are needed in order to develop reliable methods for efficiently producing stable products that can be easily delivered to food and protect sensitive ingredients from the environment as well as reacting with one another. It is also important to develop standard methods that can better compare different encapsulation techniques. Development of encapsulation specifics for food processing, proper delivery of nutraceuticals, and quality control of products is of utmost global interest and must be researched. In addition, proper packaging of encapsulated ingredients may benefit from novel ideas and further research.

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Part V

Preservation Using Heat and Energy

35 Pasteurization and Food Preservation

M. N. Ramesh

CONTENTS

35.1 INTRODUCTION

Pasteurization is one of the most important steps in preservation and is essential for food safety. It greatly improves a product's "keeping" quality by effectively destroying virtually all disease-producing and most other bacteria (source: www.fao.org). Pasteurization is a process of heat treatment to inactivate enzymes and to kill relatively heat-sensitive microorganisms that cause spoilage, with minimal changes in food properties, for example, sensory and nutritional characteristics. It is also defined as a "mild heat treatment" for avoiding microbial and enzymatic spoilage. It is used to extend the shelf life of food at low temperatures, usually 4°C, for several days (e.g. milk) or for several months (e.g. bottled fruit juice). The heating of liquid foods to 100°C is employed to destroy heat-labile spoilage organisms such as non-spore forming bacteria, yeast, and molds.

35.2 PURPOSE OF PASTEURIZATION

The primary object of pasteurization is to free the food of any microorganism that might cause deterioration or endanger the health of the consumer. The severity of the heat treatment and the resulting extension of shelf life are determined mostly by the pH of the food. In low-acid foods (pH >4.5), the main purpose is the destruction of pathogenic bacteria, whereas, below pH 4.5, the destruction of spoilage microorganisms or enzyme inactivation is usually more important. Table 35.1 shows different pasteurization conditions for food. Pasteurization does not aim to kill the spore-bearing organisms, such as thermophilic *Bacillus subtilis*, but these organisms and most other spore-bearing bacteria cannot grow in acidic fruit juices, and consequently, their presence is of no practical significance. The pasteurization of carbonated juices need only be conducted at such a temperature and for such

TABLE 35.1
Purpose of Pasteurization for Different Foods

Food	Main Purpose	Subsidiary Purpose	Minimum Processing Conditions
pH < 4.5			
Fruit juice	Enzyme inactivation (*pectinesterase* and *polygalacturonase*) (yeasts, fungi)	Destruction of spoilage microorganisms	65°C for 30 min; 77°C for 1 min; 88°C for 15 s
Beer	Destruction of spoilage microorganisms (wild yeasts, *Lactobacillus* species) and residual yeasts (*Saccharomyces* species)	--	65–68°C for 20 min (in bottle); 72–75°C for 1–4 min at 900–1000 kPa
pH > 4.5			
Milk	Destruction of pathogens; *Brucellaabortis*, *Mycobacterium tuberculosis*, (*Coxiella burnetti*)	Destruction of spoilage microorganisms and enzymes	63°C for 30 min; 71.5°C for 15s
Liquid egg	Destruction of pathogens Salmonella seftenburg	Destruction of spoilage microorganisms	64.4°C for 2.5 min; 60°C for 3.5 min
Ice cream	Destruction of spoilage microorganisms	Destruction of pathogens	65°C for 30 min; 71°C for 10 min; 80°C for 15 s

a time that yeasts and molds are destroyed. Yeast is killed by heating at 60–65°C, and the resistant mold spores, negative in most cases, are heated up to 80°C for 20 minutes. But molds require oxygen for growth, and for this reason, heavily carbonated juice can be pasteurized safely at 65°C, which destroys yeast cells. Most still (non-carbonated) juices must be pasteurized at 80°C. Juices of high acidity may be pasteurized at lower temperatures of 60–65°C.

The processing of containers of food that have a naturally low pH (e.g. fruit pieces) or in which the pH is artificially lowered (e.g. pickles) is similar to canning. In acidic products like tomatoes, mangoes, bananas, etc. (pH 4.0 to 4.4), yeast, molds, and bacteria (both thermophilic and mesophilic) grow. The main risk of spoilage is from spore-forming species other than *Clostridium botulinum* especially *B. coagulans* among the aerobes and *Clostridium pasteurianum* and *Clostridium thermosaccharolyticum* among the anaerobes. In high-acid food (pH <3.9) like pineapple juice, spoilage is generally caused by non-spore-forming bacteria (*Lactobacillus* and *Leuconostoc*), yeast, or molds. Fruits with a pH lower than 4.5 contain enzyme systems such as catalase, peroxidase, polyphenol oxidase, pectinesterase, etc., in addition to spoilage organisms. Unless inactivated, these enzymes are likely to cause undesirable changes in the canned products. Some of these enzymes, particularly peroxidases, have higher heat resistance than the spoilage organisms and have been used in evaluating the thermal processing of canned fruits.

35.3 TYPES OF PASTEURIZATION

There are several types of pasteurization: (i) In-package pasteurization: Inside packages, heating to the level of sterility is not required. A gradual change in temperatures is preferred in some containers. (ii) Pasteurization prior to packaging: Preheating is good for foods that are sensitive to high=temperature gradients. (iii) Batch pasteurization: This is also called the low-temperature-short-time process. Here fluid foods like milk are held in a tank where they are heated to 62.8˚C for 30 minutes. A batch pasteurizer consists of a steam jacket kettle or a tank equipped with steam coils in which juice or milk is heated to the desired temperature. (iv) Continuous pasteurization: This is also called the high-temperature-short-time process. Foods like milk are subjected to 71.7˚C for about 15 seconds or more by flowing through different heat exchangers. In continuous pasteurization generally plate heat exchangers, tubular heat exchangers, and scraped surface heat exchangers are used depending on the viscosity of the fluid food material. The heating medium is usually steam or water.

35.4 PASTEURIZATION TESTING

Over the past 60 years, the industry has used colorimetric tests to determine proper pasteurization. This manual inspection method is based primarily on a technician's subjective interpretation of the results. In 1990, a new rapid enzymatic assay (test) was designed to confirm pasteurization. This test involves the use of an automated instrument and a fluorometric assay. Alkaline phosphatase (ALP) is an enzyme naturally present in raw milk, which is used as an indicator for proper milk pasteurization. Non-pasteurized or raw milk contains ALP, which causes intra-abdominal bacterial infection after drinking the milk, whereas after pasteurization**,** ALP is denatured. Therefore, milk companies test the milk after pasteurization by ALP testing wherein the instrument interprets the results instead of a technician, dramatically reducing the evaluation process from 90 minutes to 3 minutes. ALP testing, unlike the colorimetric method, can be used to confirm pasteurization of many different products including bovine, sheep, and goat milk, flavored and cultured products, and cheeses. This test is revolutionizing the way the industry checks for pasteurization. Dairy processors enjoy higher precision and reproducibility and a tenfold sensitivity improvement. This enhances process improvement and troubleshooting while allowing immediate

process validation following maintenance. The ALP method has been accepted in Europe as a reference method, and dairy producers are beginning to implement the equipment. In the U.S., the test's accuracy and sensitivity resulted in the FDA lowering its pasteurization acceptance criteria from 500 to 350 mU/L of ALP activity. By lowering the criteria with better technology, plants are able to improve HACCP programs and advance the cause of food safety to protect consumers.

Biostrips have become more popular for testing of different chemical and biochemical parameters. A quick, simple, and economical test using dry-reagent strips for the detection of ALP activity in milk has also been developed [1]. It is based on ALP reaction with *p*-nitrophenyl phosphate in the presence of water to liberate *p*-nitrophenol and inorganic phosphate. *p*-Nitrophenol on reacting with a specific chromogen changes the color of the strip from light blue to green, which is visible to the naked eye. The strip has a sensitivity of >0.5 units/L. The strips may be used in dairy companies and remote areas where expensive instruments are not available. The strip is stable for more than a year at room temperature.

Processing methods have different effects on the nutritional and sensorial qualities of juices. Thermally treated juices produced by full or flash pasteurization are still the most widely marketed product. Acidophilic microorganisms have been shown to be the major contaminants of citrus juices, especially lactic acid bacteria and yeasts [2]. The redox potential (Eh) is a physiochemical parameter that determines the oxidizing or reducing properties of the medium, and it depends on the composition of the food (thiol-containing amino acids, peptides, proteins, and reducing sugars), the pH, the temperature, and for a large part the concentration of dissolved oxygen [3]. This parameter plays an important role in the cellular physiology of microorganisms such as growth capacity [4, 5] enzyme expression [6], and thermal resistance [7–9].

35.5 ACHIEVING DESIRED PASTEURIZATION

Broadly, pasteurization can be achieved by a combination of time and temperature such as (i) heating foods to a relatively lower temperature and maintaining that temperature for a longer time, e.g. holding pasteurization and pasteurization by overflow method, and (ii) heating foods to a high temperature and holding this temperature for a short time only. Pasteurization can be performed in two ways: (i) by first filling sterile containers with the product and then pasteurizing or (ii) by pasteurizing the product first and then filling in sterile containers.

35.6 PASTEURIZATION EQUIPMENT

35.6.1 Pasteurization of Packaged Foods

In packaged foods like beers and fruit juices, in-container processing is applied. When the container is glass, generally hot-water processing is used to reduce any damage due to thermal shock. After processing, the container is cooled to 40˚C, which also facilitates evaporation of the surface water. This minimizes external corrosion of metal containers or caps and accelerates the setting of adhesives used in labels. Hot-water pasteurizers may be batch or continuous in operation. The simplest batch equipment consists of a water bath in which crates of packaged food are heated to a pre-set temperature and held for the required length of time. Cold water is then pumped in to cool the product. A continuous version consists of a long trough fitted with a conveyer belt to carry containers through the heating and cooling stages [10].

35.6.1.1 Water Bath Pasteurization

For acidic food products that can be adequately pasteurized at temperatures of 100˚C or below and for non-sterile meat items, a water bath is one of the simplest methods of heating for pasteurization. The water bath may be either a rectangular steel tank or a 1-m diameter vertical retort. The product is packed in retort crates or in racks and immersed in the bath for pasteurization. Cooling may be carried out in the same tank used for heating, or the containers may be moved from the heating tank to a cooling tank. Heating and cooling may be carried out in steps. Essentially, the same procedure is followed in the processing of meats, pickles, apple sauce, and other acidic food products [11]. The continuous water bath is an improvement over batch operation and is in use by both pickle processors and fruit canners for pasteurization where high production rates are required. A conveyer belt moves through the tank at a selected speed to provide adequate time in the bath to accomplish pasteurization. The tank is usually divided into sections, each of which is heated and controlled separately. In continuous water bath pasteurizers, the jars and cans must proceed down an incline into the tank and up an incline when they come out of the tank. Since there is considerable hazard in conveying glass containers up or down an incline, plants that pasteurize glass-packed products have switched to water-spray or steam pasteurizers.

35.6.1.2 Continuous Steam or Water-Spray Pasteurizer

The continuous water-spray pasteurizer is extensively used for pasteurizing beer and acidic food products. In this type of unit, the bottles or cans are conveyed through the pasteurizer either by a walking beam or by a continuous belt conveyor. It is common practice to have as many as six different temperature zones or sections through the pasteurizer to obtain maximum efficiency. The sections are first preheat, second preheat, pasteurizing zone, pre-cooling, cooling, and final cooling zone. Water-spray units are designed so the water in the first preheat zone is the water that drains off the jars in the pre-cooling zone, and the water that is sprayed in the pre-cooling zone is used in the first preheat zone. In this way, a considerable amount of heat is recovered and reused, and a reduced amount of cooling water is required. Cooling water is also recirculated [11].

Glass containers should not be subjected to excessive "thermal shock"; when heating products in glass containers it is recommended that the thermal shock temperature difference be kept below 20˚C and under no condition to exceed 40˚C. When cooling a hot product in a glass container, temperatures

are more critical; 10°C is a desirable maximum, and under no conditions should the temperature change exceed 20°C. Several sections are necessary in both continuous-steam and water-spray pasteurizers to heat glass containers efficiently; however, metal containers may be pasteurized in the same equipment. Through the use of sectionalized equipment, it is possible to have high-temperature heating and low-temperature cooling of glass containers with a minimum amount of "thermal shock" breakage. The water-spray-type unit has been very successful in the pasteurization of beer and similar products where the operation proceeds under ideal conditions.

The steam pasteurizer is simply a tunnel that is open at both ends with a conveyer along the bottom. Cloth baffles are hung between each section, but these are not adequate to hold the steam in the pasteurizer against strong air currents. The rate of heat transfer from the steam–air mixture to the food container is not constant in the steam pasteurizer, but varies with steam temperature and steam velocity.

35.6.1.3 Tunnel Pasteurization

Hot-water sprays are used to heat containers as they pass through the different heating zones of the tunnel and provide an incremental rise in temperature until pasteurization is achieved. Cold water sprayed then cools the containers as they continue through the tunnel. Steam tunnels have the advantage of faster heating, giving shorter residence time and smaller equipment. Savings in energy and water are achieved from heat recovery from the hot products and re-circulating water. Temperatures in the heating zone are gradually increased by reducing the amount of air in the steam–air mixtures, and cooling takes place using water sprays or by immersion in a water bath [10].

35.6.2 Pasteurization of Unpacked Liquids

35.6.2.1 Long-Hold or Vat Pasteurizing

Vat or tank-type heat exchangers are used for the long-hold method of pasteurization. Here the raw product is pumped into the vat, heated to the pasteurizing temperature, held for the required time, and pumped from the vat through cooling equipment. With most vat pasteurizers, circulation of the heating medium can be started as soon as the filling of the vat is begun. In this way, some heating of the product takes place during filling so that the heating time can be shortened. With some designs of pasteurizing vats, cold water can be circulated over the outside of the inner liner as soon as the holding period is completed, thus doing part of the cooling in the vat [12]. It is considered good practice with all heat-exchange equipment for dairy products to use a heating medium (hot water or steam vapor) only a few degrees warmer than the milk, for then there is less accumulation of milkstone on heating surfaces and less danger of injury to cream line or flavor.

35.6.2.1.1 Advantages

Vat pasteurizers are well-suited for small plants and for low-volume products in larger operations. They can handle a variety of products with a wide range of physical characteristics. They are especially well-adapted to the processing of cultured products such as buttermilk and sour cream, which, in addition to being pasteurized and cooled, require mixing for the incorporation of starter, several hours of quiescent holding for incubation, agitation for breaking the curd, and final cooling in the tank.

35.6.2.1.2 Disadvantages

There are several disadvantages to consider. Vat pasteurization is normally a batch operation, and is inherently slow, although the flow can be made continuous by the use of three or more vats (depending upon the holding, heating, filling, and emptying times). The operation may even be made automatic by the use of complex and expensive controls. In the great majority of batch operations, manual controls are used, and constant attention must be given by the operator to prevent overheating, over-holding, and burning. Another disadvantage is that regenerative heating is not possible in the vat, so both heating and cooling is relatively expensive.

35.6.2.2 Heat Exchanger Pasteurizer

Small-scale batch pasteurization is carried out in open boiling pans or in scraped surface heat exchangers which are used for some liquid foods with high viscosity. Generally, less viscous liquids are pasteurized by a plate heat exchanger. Some products, such as fruit juices and wines, require deaeration before pasteurization to prevent oxidative changes during storage. This can be achieved by spraying liquids into the vacuum chamber after which dissolved air is removed [13]. The plate heat exchanger consists of a series of thin vertical stainless-steel plates. The plates form parallel channels held tightly together in a metal frame and separated by rubber gaskets to produce a watertight system. The plates are corrugated to induce turbulence for a high heat transfer rate. The advantages of heat exchangers over in-bottle processing include (i) more uniform heat treatment, (ii) simpler equipment and lower maintenance costs, (iii) reduced space requirements and labor costs, (iv) greater flexibility for different products, and (v) greater control over pasteurization conditions.

A number of systems for pasteurizing milk have been used commercially. The first were batch systems employing holding tanks. The milk was heated in a jacketed tank to a temperature of 65°C and held for 30 min. This type of system is rarely found, but it can be suitable for small operations. Improvements on the batch system came with the advent of the continuous-holding or retarding systems. Holding times and temperatures are the same; however, the tanks automatically fill, hold, and empty in a timed cycle. The system of choice in most modern dairies is now the high-temperature-short-time (HTST) process.

The heat exchanger has the following advantages over the batch and continuous-holding systems: (i) lower initial cost due to elimination of holding tanks, (ii) less labor required as the system incorporates mechanized circulation cleaning, (iii) saved space (about 10,000 liters/hr can be pasteurized in 4.5 m^2), (iv) increased flexibility (capacity of the plant and processing rate can be easily controlled), (v) ease of recording

and safeguarding the pasteurization temperature requirements (milk can be readily diverted if it does not reach minimum safe pasteurized temperatures), and (vi) lower operating costs (plant can be almost entirely automatically controlled).

The capacity of the equipment varies according to the size and number of plates—up to 80,000 liters/hr. Other types of heat exchangers are also used for pasteurization. In particular, the concentric tube heat exchanger is suitable for more viscous food and is used with dairy products, mayonnaise, tomato ketchup, and baby food. It consists of a number of concentric stainless-steel coils, each made from double- or triple-walled tubes. Food passes through the tube, and heating and cooling water are recirculated through the tube walls. Liquid food is passed from one coil to the next for heating and cooling, and the heat is regenerated to reduce energy costs. Pasteurized food is immediately deposited into cartons or bottles. Care with cleaning and hygiene is therefore necessary.

35.6.2.3 High-Temperature-Short-Time Pasteurizers

HTST pasteurizers are continuous-flow systems using tubes, plates, swept surfaces, and direct steam, in conjunction with a timing pump, a holder, and controls for temperature and flow rate. The great majority of HTST pasteurizers use plate-type heat exchangers with sections for regenerative heating and cooling. A schematic of a plate-type HTST pasteurizer is shown in Figure 35.1. This is also referred to as a flash pasteurizer [12]. Continuous pasteurizers assure that all the product of an entire run receives uniform treatment. HTST pasteurizers employing regenerative heating are much more economical to operate than batch pasteurizers. In the application of controls, the general requirements for flow rate, temperature, and pressure must be considered; these are the factors that govern proper operation and public health safety.

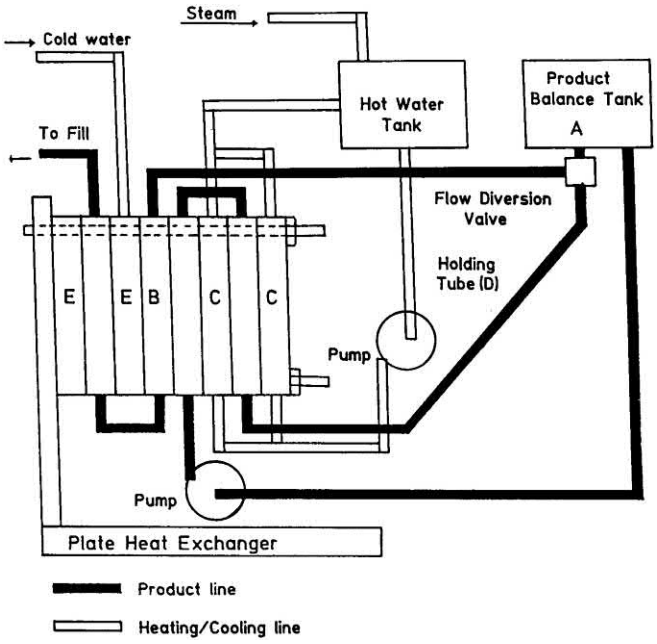

FIGURE 35.1 Plate heat exchanger pasteurizer.

The flow rate through a continuous pasteurizer is regulated by metering or a timing pump. A positive displacement pump of the rotary or piston type is used almost exclusively for milk and milk products. Often variable speed drives are employed so that the flow rate can be changed when desired. A continuous pasteurizer must include synchronization of holding time and flow rate. Controlling temperature includes maintaining a uniform product temperature at some set value at or above the legal minimum, and diverting the flow, directing it back through the system if, at the end of the holder, it is below the legal minimum temperature. Usually, a safety thermal limit-recording controller is used which keeps a continuous record of the temperature.

The pressure is especially important in two areas of continuous pasteurizers—the regenerator and the flow-diversion valve. Where product-to-product regeneration is used, it is necessary, for public health reasons, to maintain at least 7 kPa more pressure on the pasteurized side than on the raw side so that any leakage through the heat-exchange can be identified, thus eliminating the possibility of contaminating the pasteurized product. In order to prevent the mixing of air into the product and inefficiency of the pump due to air leakage into the system, the entire system is operated at a positive pressure (above atmospheric pressure). A centrifugal booster pump is employed between the product storage and the regenerator. This will ensure that the pasteurized product is always under higher pressure than the raw product.

To facilitate this, it is necessary to (i) size the booster pump correctly to deliver the rated capacity at a predetermined pressure and (ii) equip the booster pump with a pressure-actuated switch located at the outlet of the pasteurized regenerator set so that the pump can run only when the pressure is at least 7 kPa greater than that on the raw-product side. If the cooler section does not produce enough back pressure on the pasteurized regenerator to satisfy the minimum 7 kPa or greater difference, it may be necessary to install a restrictor in the line. The other pressure requirement is that which is needed on the diverted milk line, since it may affect the holding time during diversion. If, upon testing, the holding time during diverted flow is shorter than that in forward flow, a restricting orifice should be placed in the diverted line.

35.6.2.4 Flash Pasteurization

The process of heating fruit juices for only a short time at a temperature a few degrees higher than the pasteurization temperature of the juice is called flash pasteurization. In this method, the juice is heated rapidly for about 1 minute to a temperature about 5°C higher than the pasteurization temperature, and filled into containers, which are sealed airtight under cover of steam to sterilize the seal and then cooled. This process can also be used for orange juice, apple juice, grape juice, etc. The advantages of this process are that it (i) minimizes flavor loss, (ii) aids in the retention of vitamins, (iii) effects economy in time and space, (iv) helps to keep the juice uniformly cloudy, (v) heats juice uniformly, reducing cooked taste to a minimum, and (vi) by this beneficial enzyme inactivation is obtained in addition to the destruction of viable microorganisms.

35.6.2.5 Deaerator and Flash Pasteurizer

Freshly extracted and screened juices contain an appreciable quantity of oxygen, which should be removed before packing. The special equipment used for this purpose is called a deaerator. The deaerated juice is then heated in flash pasteurization equipment. Commercial deaerators and flash pasteurizers differ greatly in design, construction, and capacity. The deaerator and flash pasteurizer have been used successfully in the case of fruit juices, like tomato and pineapple and orange.

35.6.2.6 Ultra-High-Temperature Pasteurizers

The equipment for ultra-high-temperature (UHT) pasteurizers is much the same as for HTST units. The controls are similar, but the operating temperature points are higher. The holder is, of course, much smaller for minimum pasteurizing time. Generally, with a holding time in the order of 3 seconds, it is impossible to determine the holding time accurately by tests similar to those used with HTST pasteurizers, and calculated holding times are preferred [12]. Ultra-high-temperature treatment is desired because of its greater bacterial destruction and its beneficial effects on body and texture in ice cream, and where confusion exists regarding requirements for UHT pasteurization, a UHT treatment may be given following regular pasteurization. This may be accomplished with a direct-steam heater installed downstream from the flow diversion valve or with steam-vacuum flavor treating equipment.

35.6.2.7 Vacreator

The vacreator is a special type of pasteurizing apparatus used particularly in the butter industry. The product is fed into a steam-heated chamber where it is flashed at a temperature of 90–96°C under 13.5–30 kPa of vacuum. It then passes into a second chamber of higher vacuum (50–67 kPa) and is reduced to a temperature of 72–82°C. From here, it passes into a third chamber of high vacuum, 91–95 kPa of vacuum, and is reduced to a temperature of 38–46°C. This process is claimed to be very effective in pasteurization and at the same time removes undesirable odors and flavors. It also employs a continuous-type machine and is especially adapted for use on high-viscosity material, although it will also operate on plain fluid milk [12].

35.7 QUALITY OF PASTEURIZED FOODS

Pasteurization is a relatively mild heat treatment, and even when combined with other unit operations (for example irradiation and chilling) there are only minor changes to the nutritional and sensory characteristics of most foods. However, the shelf life of pasteurized foods is usually only extended by a few days or weeks compared with several months with the more severe heat sterilization methods.

In fruit juices, the main cause of color deterioration is enzymatic browning by polyphenoloxidase. This is promoted by the presence of oxygen, and fruit juices are therefore routinely deaerated prior to pasteurization. The difference between the whiteness of raw milk and that of pasteurized milk is due to homogenization, and pasteurization has no measurable effect. Other pigments in plant and animal products are also unaffected by pasteurization. A small loss of volatile aroma compounds during pasteurization of juices causes a reduction in quality and may unmask other cooked flavors. Volatile recovery may be used to produce high-quality juices, but this is not routinely used. The loss of volatiles from raw milk removes a hay-like aroma and produces a blander product.

In fruit juices, losses of vitamin C and carotene are minimized by deaeration. Changes to milk are confined to a 5% loss of serum proteins and small changes to the vitamin content (Table 35.2). Most of the pasteurized food products have a low pH either due to the low natural pH of the system or because the product has been fermented to produce an acidic environment. Since most of the heat-labile nutrients are relatively stable in acidic conditions, nutrient losses in those products are relatively minor. Although thermal losses during pasteurization may be small, oxidative losses can be high. Thus pasteurization of liquid foods such as fruit juices, beer, and wine is generally accomplished in indirect heat exchangers like plate or double tune rather than open film-type pasteurizers. Often fluids are deaerated prior to pasteurization. The most important nonacid liquid food is milk. The effect of pasteurization on the nutrients of milk has received considerable attention.

Ascorbic acid degradation of single-strength orange juice was lower than that of fully pasteurized orange juice under aerobic conditions with ca. 23% and 83%, respectively, after 7 weeks of storage. It is reported that unpasteurized orange juice exhibited significantly higher ascorbic acid retention than fully or lightly pasteurized juice during storage at 4°C for 36 days [14]. Similar results have also been reported that long-life commercial orange juice, when stored in open containers in the refrigerator for 1 month, lost 60–67% of its ascorbic acid, whereas fresh orange juice lost 7–13% [15]. These differences in the destruction rates of ascorbic acid during storage between single-strength and fully pasteurized orange juice may be due to (i) oxygen consumption by natural background flora in single-strength juice, (ii) the thermal oxidative destruction of several protectors of ascorbic acid such as bioflavonoids (anthocyanins, flavonols, etc.), vitamins P1 and P2, vitamin E, and phenols, and (iii) the production of oxidative molecules during the first steps of the Maillard reaction. The ascorbic acid content of single-strength orange juice was found to be 41 mg/100 ml, and this decreased during

TABLE 35.2
Vitamin Losses during Pasteurization of Milk

	Method of Pasteurization	
Vitamin	**HTST**	**Holder**
Vitamin B_6	0	0
Thiamin	6.8	10.0
Vitamin C	10.0	20.0
Vitamin B_{12}	0	10

Source: Ford et al. [38].

pasteurization at 90°C/1 min to 38 mg/100 ml. The ascorbic acid degradation rates of single-strength and fully pasteurized orange juice were high for the first 3 weeks of storage; they then stabilized up to the 7th week.

The ungassed juice sample exhibited higher browning than both other conditions (nitrogen and gas mixture) for $P \leq 0.35$. Fully pasteurized orange juice, gassed by nitrogen or the gas mixture, then stored under the same gas, showed a significantly lower browning index than that of un-gassed juice (control) ($P \leq 0.25$). Oxygen elimination in the gassed samples (nitrogen and gas mixture) could decrease the evolution of browning because browning is considered as an oxidative reaction [16]. Furfural formation, which is due to the decomposition of ascorbic acid, has a similar effect to sugar and may combine with amino acids and contribute to juice browning. Storage temperature can also affect the evolution of browning. It is reported that 12°C was the critical storage temperature for furfural accumulation [17].

The pH levels of different samples of orange juice (uninoculated with the test microorganisms) were monitored during ascorbic acid and browning experiments. The pH value significantly decreased for control-unheated samples. The consumption of oxygen by natural background flora and the production of acidic metabolites may account for this decrease in pH values. Storage under both oxygen-free and reducing conditions improves the microbial and organoleptic quality of pasteurized orange juice. Consequently, it might be proposed to adjust the redox potential by gas just after heat treatment in order to maximize thermal destruction of microorganisms and to stabilize the product during storage [18].

A comparative study between the aromatic profile in fresh orange juice versus deaerated and pasteurized juices, respectively, was conducted in order to understand the evolution of volatile components after deaeration and pasteurization processes. The aromatic fractions isolated by simultaneous distillation and extraction are analyzed by capillary gas chromatography–mass spectrometry. At the qualitative level, all the volatile components in fresh orange juice were also found in the counterparts after deaeration and pasteurization processes. According to statistical analyses, significant losses in the concentration of volatile components occurred during the deaeration process, while there were no statistically significant differences determined among concentrations of volatile components in deaerated and pasteurized juices. These results show that during the industrial processing of orange juice the biggest losses in the concentration of volatile components occur during deaeration. The pasteurization process does not change the analytical composition of deaerated orange juice in a significant way for any of the 42 quantitated compounds [19].

Changes in carotenoid pigment content and juice color due to thermal pasteurization of Valencia orange juices have been studied [20]. Total carotenoid pigment content loss was significant ($P < 0.05$) after thermal pasteurization at 90°C for 30 s. Thermal effects on carotenoid pigment contents, especially on violaxanthin (–46.4%) and antheraxanthin (–24.8%), were clearly observed. With the loss of violaxanthin and antheraxanthin, lutein became the major carotenoid, followed by zeaxanthin, in pasteurized Valencia orange juice. There was perceptible color change after orange juice pasteurization, which led the juice color to become lighter and more saturated. Decreases in CIE a* value and increases in CIE L*, b*, h*, and C* are the major color changes after pasteurization. Overall increases in reflected light might also influence the perception of color to a great extent in pasteurized orange juice. Total color difference (DE*) compared to the fresh juice was 2.92 ± 0.97 ($P < 0.05$).

35.8 PACKAGING OF PASTEURIZED FOODS

Both bottles and cartons take into account the properties of milk and provide packaging acceptable to consumers worldwide. Glass bottles have the advantage of being easily cleaned, transparent, and rigid, but have the great disadvantage of high weight and fragility. Increasingly, milk is also packaged in gable-top (PurePak, Elopak) or other carton types (TetraBrik). Even though the equipment may be expensive to install, the advantages include a lower price per unit of milk and a lower risk of contamination from the air during filling [21]. Smaller quantities have been packaged in plastic pouches. A cylindrical milk carton with a reclosable pouring lid has been introduced [22]. While suitable for sterilized milk, glass bottles are a problem for "long-life" milk. The question of the container is therefore of vital interest to the dairy industry, as about 50% of all milk produced is sold in liquid form [22]. Sunlight can destroy riboflavin and vitamin C in milk, producing a taint by the oxidation of the fat [22] and protein. This led to the use of brown glass bottles, which hold back the light rays responsible. However, taint is very rare, brown bottles are not very attractive, and it has also been found that milk becomes sour faster in brown than in colorless bottles [23].

35.8.1 Returnable Bottles

For economic reasons, the use of returnable glass bottles has continued over many years. The glass bottle will take a long time to disappear because of its economic advantage, the traditions of the industry, and the attitude of the consumer. Other factors such as transport costs have led to the use of non-returnable packaging, although more recently, "green" considerations have led to the re-introduction of returnable bottles [22–24]. The advantages of non-returnable containers are (i) elimination of returned empties, (ii) elimination of collecting, sorting, and washing, (iii) elimination of the foreign object problem, (iv) elimination of the glass fragment problem, and (v) reduction in transport costs. The disadvantages are (i) possible increases in costs of packages, (ii) lack of consumer acceptance, (iii) delivery problems resulting in lower total sales, (iv) hygiene problems, and (v) environmental considerations. Plastic containers and plastic-coated cartons are nearly sterile by virtue of their method of manufacture. No sterilizing process is necessary for pasteurized milk, but for the aseptic filling of milk, sterilization is essential. So far, TetraBrik has proved the most effective. Containers for this purpose must be sterilized immediately before filling.

35.8.2 Glass Bottles

The traditional glass bottles used for fruit juices and fruit juice beverages provide many advantages. Glass is not susceptible to mold growth and is impermeable to odorous vapors and liquids. Hot-filling and in-bottle pasteurization are generally employed for pure fruit juices or products that do not contain preservatives. Hot-filling is achieved by passing the liquid product through a heat exchanger and then filling above 70˚C. The closure is then applied. Any microbiological contamination on the inner surfaces of the bottle and the closure is destroyed by hot liquid, and adequate sterility is obtained without heating the container [22]. Glass bottles can also be covered with a polystyrene shield, which enables bottles to be reduced in weight without risking breakage of bottles. Sleeves give protection, and graphics can be added easily. Some bottles are shrink-wrapped with plastic sleeves.

35.8.3 PET Bottle and Other Plastic Containers

PET bottles are displacing those made from PVC for products such as edible oils and mineral waters, as well as glass bottles for carbonated products. Improvements in processing technology have resulted in the appearance of stretch-blown PVC bottles. PET bottles have also become even lighter than before (a 2 L bottle usually weighs less than 40 g) and can be coated externally with PVC to provide improved resistance to gas permeability. Polyethylene and PVC bottles are being used for squashes and cordials, but shelf life is restricted compared to glass [25]. Unlike glass, the PET bottle will lose carbon dioxide with time, about 15% over 4 months [26, 27]. Hence PET is preferable for drinks with high carbon dioxide content in large bottles, whereas for lower carbonation levels, and small- or medium-sized bottles, other materials may be better. Other forms of plastic container have also been used [25, 28], for example, the Plasto-can, a coextruded plastic container with conventional aluminum easy-open can ends, or the the Rigello container, a multilayered polypropylene foil extrusion with a spherical bottom and tear-off cap assembled in a paperboard cylinder. New combinations of materials in can form are also being developed. Coca Cola has patented a PET/aluminum can with easy open top [29]. High barrier plastics cans, which can be recycled, are under investigation. Orange juice has also been packed in clear oriented polypropylene bottles, which provide good oxygen and moisture barrier properties [30]. Tamper-evident pull-tab closures are used on this container. Paperboard basket carriers, plastic clips (on bottlenecks) and shrink films are used to provide multipacks holding three, four, or six units.

35.8.4 Cans

Fruit juices and fruit juice concentrates are frequently distributed in cans [31]. The most common are standard tinplate containers, but specially lacquered and coated cans are also used, especially for high-acid products. Cans are usually hot-filled, but sometimes are aseptically filled. Cold-filling after pasteurization is occasionally employed, but refrigerated or frozen storage is then advisable. Products preserved with benzoic acid can also be filled cold after pasteurizing, but sulfated products are incompatible with cans. The juice tends to deteriorate in the cans due to corrosion and an increasing amount of tin and iron in the product.

In the normal hot canning process, the juice is first deaerated to improve its flavor stability and then pasteurized to destroy microorganisms and to inactivate enzymes. After hot-filling into the cans, the lids are applied and sealed immediately before cooling, which forms a slight vacuum in the headspace as the liquid contracts. This is desirable as the presence of oxygen encourages corrosion (cold-filling operations usually involve undercover gassing, in which the headspace is replaced by carbon dioxide immediately before sealing the lid).

Carbonated beverages are susceptible to metal pick-up and are therefore packaged in lacquered two-piece aluminum cans or three-piece tin-plate with side seams having a special tab design to withstand the internal pressure. Warming the filled cans immediately before packaging is important; otherwise, the cans when filled with cold carbonated liquid attract a layer of condensation from the atmosphere and may corrode on the outside. Frozen orange juice concentrate has been distributed in composite paperboard or plastics canisters of approximately 170 ml capacity [25]. There are many pack variations, including canisters with tear-off ends or, frequently, leaving the product unpasteurized to provide maximum freshness of flavor. Spoilage may result if it is left unfrozen.

Bulk frozen orange juice is packed in 200 L polyethylene drums or polyethylene-lined steel drums or transported in tankers. However, aseptically produced juice (e.g. in bag-in-box systems) is replacing bulk frozen juice. Beverage cans are also sold in [32] multipacks of four, six, or more. The most common form of over wrapping which assists handling and distribution is a plastic ring carrier which slips underneath the rim of the can and grips tightly throughout distribution. Paperboard multi-packs are also popular as well as shrink films.

35.8.5 Cartons

Pasteurized fruit juice and soft drinks can be packaged very successfully in cartons with a polyethylene coating or in plastic containers [32]. These products have a limited shelf life when stored in a refrigerator. Materials selected must not absorb flavor components from the juice. In addition, acid diffusion into the plastics material can delaminate the package. Polyethylene is the most common surface-contact material and is regarded as chemically stable with most food products. Packaging materials must also provide the best possible barrier to light as light affects the color and nutritive value of fruit juices. Aseptic filling of fruit juices and other drinks into TetraPaks and other systems (e.g. Combibloc, PurePak, Elopak) has also become popular, giving the product an extended shelf life. Such products have advantages over hot-filled products or non-aseptically packaged products, which need a chilled distribution chain [33].

TABLE 35.3
Steam and Electrical Requirements of a Pasteurizer

Percent regeneration	80	85	90	95
Steam (kg/L)	0.012	0.01	0.005	0.003
Power (kWh/1000 L)				
Pumping	0.66	0.88	1.54	2.86
Refrigeration Cooling	3.52	2.64	1.76	0.88
Primary Energy (MJ/L)				
Steam	0.072	0.053	0.034	0.021
Electricity	0.059	0.050	0.047	0.053

Sources: Upadhya [35]; Harris [36].

35.9 ENERGY ASPECTS OF PASTEURIZATION

Indirect heating through a heat exchanger involves much more energy than direct heating with infusion heating. To reduce the energy input and hence improve the efficiency of the system, a regenerative method is adopted. For example, total energy difference between the 90 and 80% regeneration capacity of a system processing milk is about 62, 131 kcal/hr. This shows that even a 10% increase in regenerative efficiency can save considerable energy [34]. Regeneration of heating and cooling streams is now an accepted conservation technique.

The heat energy consumption for pasteurization will be about 30 MJ per 1000 L milk, and correspondingly the cooling energy is about 4 kWh per 1000 L. Normally, pasteurizers will have the facility of regeneration, with an efficiency of 75 to 92%. Efficiency can be increased by increasing the number of plates in the regeneration section. But this increases the pumping pressure and hence the electrical energy. Hence, the maximum regeneration is about 90%. Table 35.3 gives the steam and electrical requirements of a large-size pasteurization unit [35, 36]. Increasing regeneration decreases the steam and refrigeration requirement but increases the electricity required for pumping. These energy requirements have also been converted into their primary fuel equivalents. This facilitates the checking of the processes on a uniform energy basis. The processing, storage, and distribution of pasteurized milk requires an estimated 2200 kJ/kg of milk [37, 38].

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36 Canning and Sterilization of Foods

M. N. Ramesh

CONTENTS

36.1 INTRODUCTION

Sterilization is the complete destruction or elimination of all viable organisms in/on a food product being sterilized. Sterilization destroys yeasts, molds, vegetative bacteria, and spore formers and allows the food processor to store and distribute the products at ambient temperatures, with extended shelf life. Sterilization procedures involve the use of heat, radiation, or chemicals, or physical removal of cells. The sterilization process consists of four distinct stages. First, the product must be heated to a temperature of 110 to 125°C to ensure sterilization. After this, the product requires a few minutes to equilibrate, since the surface will be hotter than the central portion of the container causing a temperature gradient. The equilibration stage allows a reduction in the temperature gradient. Next, the product must be held at this temperature for a certain period of time to ensure a predetermined sterilization value designated by F_o. Finally, the product has to be cooled, mainly to arrest further heat treatment and to avoid overcooking [1]. The basic principles of sterilization technology as applied to food processing are [2]:

- The processed product must be free from microorganisms capable of producing food poisoning toxins and those microorganisms which cause food spoilage during product shelf life, until it is consumed.
- *Clostridium botulinum* spores are capable of growing in low-acid (pH >4.6) products during storage and hence must be heat treated to the equivalent of at least 121.1°C for 3 minutes (an F_o value of 3) to achieve a 12-decimal reduction of the microorganism.
- The processing conditions should be applied to the slowest-heating point referred to as "cold point." This facilitates the assumption that, when the slowest-heating part is sterilized, by exposing it to the required time–temperature profile, the rest of the product will be sterilized.

Practically, complete sterilization will lead to deteriorations in product quality and nutrients [3]. Hence, in practice, commercial sterility is targeted. Commercial sterility is defined as a product that has been optimally processed so that under normal conditions, the product will neither spoil nor endanger the health of the consumer and also retain its organoleptic properties and nutrients [4]. The pH of the product is an important factor in determining the severity of the sterilization process.

36.2 THEORY OF STERILIZATION

Thermal treatment of food products to render them free of pathogenic microorganisms has been practiced for several years. However, a method to quantify the microbial destruction that takes place during a thermal treatment has only been understood for the last 75 years. To determine the amount of microbial destruction that a thermal treatment delivers to a process requires an understanding of both the amount of heat delivered to every portion of the food product and the destruction kinetics of the microorganisms of interest. The amount of heat delivered by the sterilization process is dependent on the way in which the product is heated and on its physical nature. Process-dependent factors include processing equipment design, type of heating media, container, or food size and shape, product composition, and viscosity (conduction or convection heated). The thermal destruction kinetics of microorganisms or their ability to be killed within the food matrix is dependent on a number of factors. These factors include pH of the product, levels and types of preservatives, water activity, the previous growth conditions of the microorganisms of concern, product composition, and competitive microorganisms [5].

The two types of bacteria of concern in food preservation are organisms of public health significance and spoilage-causing bacteria. In low-acid foods with a pH greater than 4.6, the organism of public health significance is *Clostridium botulinum*. Canned foods are processed based on the survival probability for *C. botulinum* of 10^{-12} or one survivor in 10^{12} cans. The organism most frequently used to characterize low-acid food spoilage by mesophilic spore-formers is PA 3679, a strain of *C. sporogenes*. Most food companies accept thermal inactivation of 10^{-5} for mesophilic spore-formers and 10^{-2} for thermophilic spore-formers. The processing time depends on the bioburden of the most resistant bacteria in a particular food, the spoilage risk involved, and whether food can support the growth of potential contaminating bacteria [6]. Although a lot of research work has been carried out on the influence of different factors on the processing time and the corresponding sterilization value, a number of uncertainties still exist on the application of these factors to scientifically arrive at the exact processing conditions. These uncertainties have been discussed in detail [7]. To avoid any risk due to these uncertainties, a safety factor is added to increase the processing time to completely sterilize the food product, which invariably reduces the nutrient content and increases the energy cost.

36.3 METHODS OF STERILIZATION

The food sterilization methods are divided into two categories: sterilization by heating (thermal processing) and sterilization without heating (non-thermal processing). Thermal processing is widely practiced in spite of some problems such as that the process of heating might reduce nutrition or deteriorate the quality of foods and that it is ineffective against certain types of bacteria. Thermal processing is further divided into two categories: in-container sterilization (bulk canning) and aseptic

sterilization (processing). The principles involved in the thermal sterilization of foods remain the same for both methods.

36.4 BULK CANNING

36.4.1 Introduction

The thermal processing operation requires the heating of food products. For a low-acid food product (pH >4.6), the product is heated to temperatures above 100°C usually in the range 115–130°C for a time sufficient to achieve a 12-log reduction of the spores of *Clostridium botulinum* as defined in Department of Health Code of Practice No.10. Current practice is, however, to move to even higher temperatures and consequently shorter process times to maximize the organoleptic and nutrient retention within the product. The time–temperature procedure required to render a product commercially sterile must be carefully determined using established procedures. Canned foods might be described as full-moisture, ambient-temperature stable food products regardless of the package form employed.

36.4.2 Processing Equipment

The food processing industry produces a wide range of products in a variety of containers. This requires an equally wide range of processing techniques, retort designs, and operating procedures. Retorting systems can be subdivided in several ways.

36.4.2.1 Methods of Processing the Containers

Two types of retorts are batch retorts and continuous retorts. In batch systems, the retort is filled with product, closed, and then put through a processing cycle. In continuous retorting systems, containers are continuously fed into and out of the retort. Batch retorts are available in a number of configurations for various applications including static, rotary, steam heated, and water heated with or without air overpressure. The air overpressure is necessary to maintain the integrity of the containers during retort operating cycles for glass and flexible containers.

36.4.2.2 Methods of Heating Medium

The heating media used in retort are steam, steam/air, water, direct flame, or fluidized bed.

36.4.2.2.1 Saturated Steam

Latent heat is transferred to food when saturated steam condenses on the outside of the container. If air is trapped inside the retort, it forms an insulating boundary film around the cans, which prevents the steam from condensing and causes underprocessing of the food. It also produces a lower temperature than that obtained with saturated steam. It is therefore important that all air is removed from the retort by the incoming steam using a procedure known as venting [8]. After sterilization, the containers are cooled with water. Steam is rapidly condensed in the retort, but the food cools more slowly and the pressure in the containers remains high. An overpressure of air is therefore used to prevent strain on the container seams (pressure cooling). When the food has cooled to below 100°C, the overpressure of air is removed and cooling continues to approximately 40°C. At this temperature, moisture on the container dries to prevent surface corrosion, and label adhesives set more rapidly. Rigid polymer trays heat more rapidly than conventional container owing to their thinner cross-section. Trays are processed in conventional equipment using saturated steam at 121°C.

36.4.2.2.2 Hot Water

Foods are processed in glass containers or flexible pouches under hot water with an overpressure of air. Glass containers are thicker than metal cans to provide adequate strength, and this, together with the lower thermal conductivity of glass, results in slower heat penetration and longer processing times than for cans, and there is a higher risk of thermal shock to the container. Foods in flexible pouches heat more rapidly owing to the thin cross-section of the container. This enables savings in energy and causes minimal overheating near the container wall. Liquid or semi-liquid foods are often processed horizontally to ensure that the thickness of food is constant across the pouch. Vertical packs promote better circulation of hot water in the retort, but special frames are necessary to prevent the pouches from bulging at the bottom, which would alter the rate of heat penetration and hence the degree of sterilization achieved [8].

36.4.2.2.3 Flames

High rates of heat transfer are possible at flame temperatures of 1770°C. The consequent short processing times produce foods of high quality and reduce energy consumption by 20% compared with conventional canning. Each can is scanned by an infrared controller after processing, instead of the usual procedures. High internal pressures (275 kPa at 130°C) limit the application of this method to small cans [8].

36.4.3 Description of Processing Equipment

A variety of retorts which use "pure" steam as the processing medium, i.e., steam free of air, are available. Steam retorts with batch container handling are vertical and horizontal still retorts, crateless retorts, and agitating retorts. Steam retorts with continuous container handling include continuous rotary sterilizers and hydrostatic retorts. Retorts operating procedures must ensure that a uniform processing temperature is achieved and maintained throughout the location of containers during the process.

36.4.3.1 Batch/Still Retorts (Horizontal and Vertical)

Batch steam retorts are usually arranged either vertically (Figure 36.1) or horizontally (Figure 36.2) and are used for canned products which are placed into baskets immediately after seaming, and are then placed inside the retort shell. The retort is made out of a metal shell pressure vessel which is fitted with inlets for steam (A), water (B), and air (E) and has

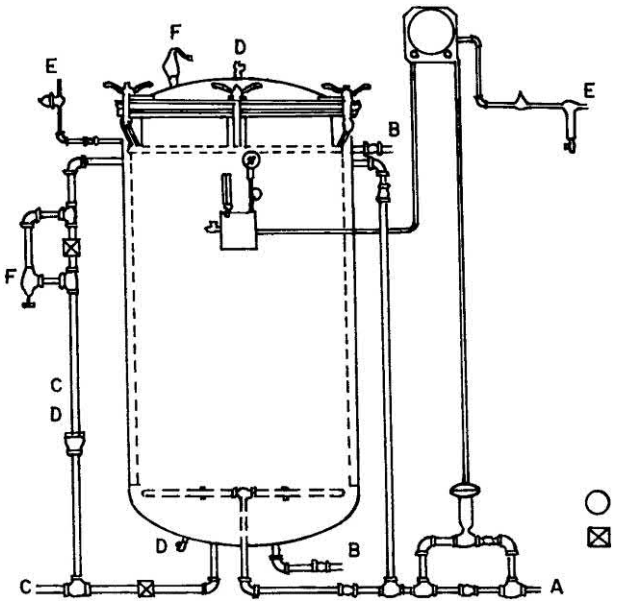

FIGURE 36.1 Vertical batch retort. (From Paine and Paine [102].)

outlet ports for venting (D) air during retort come up, and for draining (C) the retort at the end of the cycle. A pocket for a thermometer or temperature-recording probe/sensor and pressure gauge is located on the side of the vessel. To ensure adequate steam movement around the temperature sensors, the pocket is fitted with a constant steam bleed (D). On vertical retorts, the lid is hinged at the top and secured to the shell during processing by several bolts. In horizontal steam retorts, the door is usually on the end of these machines, which can swing open. A safety valve and pressure relief valve (F) are also provided for safety of the equipment [9].

The operating cycle for this type of retort involves loading the sealed containers into the retort, bringing the retort up to a temperature of around 100°C, and then allowing steam to pass through the vessel to the atmosphere for sufficient time so that all air in the retort and between the cans is removed (venting) before the retort is finally brought up to the operating pressure and processing temperature. At the end of the processing time, the steam is turned off and a mixture of cooling water and air introduced into the retort to cool the cans. The purpose of the air is to maintain the pressure in the retort following the condensation of the residual steam after the initial introduction of cooling water. If this pressure is not maintained the containers may deform due to pressure imbalances between the internal pressure in the cans and the retort. As the temperature drops, the pressure in the retort may be controlled and gradually reduced until atmospheric pressure is reached and water can be allowed to flow through the retort, cooling the cans to a temperature of about 40°C before they are removed from the retort. Cans are removed from the retort at this temperature since this allows the surface of the cans to dry rapidly by evaporation, so reducing the risk of leaker spoilage [10]. The water is preferably sprayed, or alternatively the retort may simply be filled and allowed to stand for sufficient time for the cans to cool to 40°C before unloading the containers.

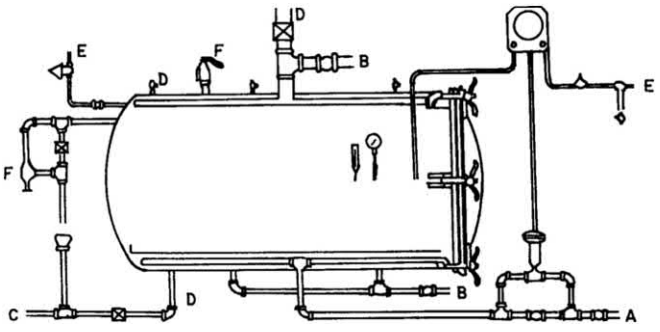

FIGURE 36.2 Horizontal batch retort. (From Paine and Paine [102].)

Both systems are static in operation. For other types of product, it is possible to assist the rate of heat penetration by agitating the cans in the steam environment by rotation either about the horizontal axis in a horizontal retort or by rotation in the vertical plane in a vertical retort.

36.4.3.1.1 Steam/Air Retort Systems

The use of glass and plastic containers has increased the use of alternative retorting systems. With these types of containers, it is usually not sufficient to rely on the strength of the containers alone to counteract the build-up of internal pressure during heating, but a constant overpressure of air is required to ensure the integrity of the package during heating. Thus the heating medium used in this type of retort is often a mixture of steam and air in proportions designed to provide the necessary steam temperature and air overpressure to maintain the package integrity. In order to ensure adequate mixing of the steam and air these retorts are fitted with a fan system to disperse the steam and air, to eliminate the development of cold spots in the processing chamber [9].

Control of this type of retort system can be difficult, particularly in ensuring an adequately uniform temperature distribution in the retort environment when the steam is being mixed with cold compressed air. Here, unlike in the case of saturated steam retorts, the presence of air must not permit a reduction in the partial pressure of the steam and hence retort temperature, but only provide the overpressure needed to ensure package integrity. However, the steam and air must be intimately mixed so that pockets of cold steam/air mix do not form in the retort and lead to inadequate processing of the cans.

Three major classifications of steam/air retorts may be identified on the basis of methods used to mix and circulate the gaseous media: air makeup, positive flow, and forced convention. In steam/air processing, heat is supplied primarily from the latent heat of condensing steam, in contrast to sensible heat transfer in superheated water systems. As a result, it is essential to have a homogeneous steam/air mixture reach all product locations. Since air is present during the process, it is unnecessary to purge all of the air from the retort by venting prior to the holding period. However, a venting procedure is advantageous for initial heating of the retort shell, retort cars, and product support racks, as well as for providing faster heating to low viscosity, convention-heating products.

36.4.3.1.2 Air Makeup Systems

These are designed such that after the desired temperature and pressure are reached, small valves along the top of the retort are left open to provide continuous venting of the retort during the heating period. The venting results in the pressure dropping to less than the set point; this causes an air makeup valve to open to reestablish the retort pressure. As air enters the retort, the temperature tends to decrease; this signals for the addition of steam, which causes a further increase in pressure. Because of the repeated deviation from the temperature and pressure set point conditions, it may be difficult to maintain stable operating conditions using this methodology. Additionally, the addition of steam and air independently may produce a nonhomogeneous heating medium. In one system activation of the air makeup valve also activated the steam valve such that steam and air, mixed outside the retort in the pipe connected to the spreader, flowed into the retort in response to a pressure drop, but this also could result in a pressure overshoot.

36.4.3.1.3 Positive Flow Systems

These are designed to improve retort control and stability by controlling pressure and temperature independently. A temperature controller operates a proportional valve on the steam line, adding steam to the retort and to maintain the set temperature, while a pressure controller for the air inlet and vent lines maintains the retort pressure. A drop in the pressure to below the set point causes the air supply valve vent to open and the vent valve to close. When the pressure exceeds the set point, the opposite occurs and the retort is vented. During processing, a constant flow of air is added to the retort, thereby causing the pressure to exceed the set point and the proportional flow vent valve to remain partially open during the process. Since steam and air are constantly vented under these conditions, temperature control would require the steam valve to remain partially open. Consequently, a continuous flow of steam and air would pass through the vessel to create a homogeneous mixture throughout the retort.

36.4.3.1.4 Forced Convection Systems

In contrast, these utilize a powerful fan to circulate the heating medium through the retort and maintain a uniform mixture of steam and air throughout the vessel. During this process, steam is added to replace that which has condensed in heating the load, and, theoretically, after the process is established there should be no need to add air to the vessel until the cooling cycle begins [11].

36.4.3.2 Water-Processing Retorts

This system is mainly used for the processing of glass jars. Raining water techniques (Figure 36.3) require the use of either an external steam injection system or heat exchanger system outside the direct environment of the retort. In the latter case, the cold water feeding the system is combined with the recycled heating medium and raised to the temperature required in the retort before being admitted to the sterilization chamber through a spray arrangement. The containers are arranged to allow good contact between the hot water heating medium and the product either using spacer bars or distribution plates. It is imperative that a good distribution of the water occurs as otherwise stratification may occur and certain containers will receive inadequate heating. Control of the temperature in this system is difficult, but the safest practice is to base the thermal process received by the product on the outlet temperature of the retort, i.e., the temperature measured in the return line to the heat exchanger [9]. The velocity of water in these retorts when passing over the packages is of vital importance as this will influence the rate of heat transfer to the product due to its effect on the heat transfer coefficient. This is unlike saturated steam retort processes where the heat transfer coefficient can be considered infinite [11].

36.4.3.3 Crateless Retort Systems

The vertical retort has grown in size, given up its crates, and become automated. These retorts are large and without crates and have been recognized as a universal symbol of low-acid food processing. They are usually 2.5 m high and 2 m in diameter with four to five times greater capacity than the conventional three-basket vertical retort. The crateless retort is filled with water, which acts as a cushion for cans filled from an automatic conveyor; after loading with cans a hydraulic lid is closed. Steam is admitted through the top opening, and this forces the water out of the retort through the bottom opening. The hot water can be recycled in another retort or in the next cycle. After processing, cooling water is let in through the bottom and is discharged through overflow. After the cooling

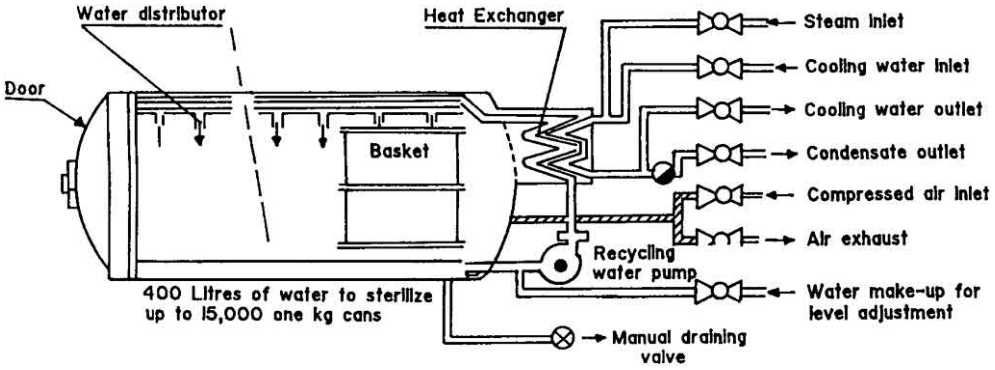

FIGURE 36.3 Water-processing retorts. (From Richardson and Selman [9].)

cycle, the retort is drained off and the bottom door is partially opened and the cans fall onto a shaker screen and are conveyed by belt to the unscrambler [12, 13]. It is critical that the steam condensate be removed from the bottom of the crateless retorts with top steam entry.

36.4.3.4 Agitating Retorts

There have been a number of different batch-agitating retorts designed to provide product agitation by rotating the containers end over end or side over side. The one most used is the Orbital Rotary Pressure Stabilizer (FMC Corp., Madera, Calif.), also known as the Orbitort. The unique design of the orbital sterilizers allows for the simultaneous loading and unloading of containers by a reel and spiral assembly. This assembly consists of an inner reel with steps attached to a drum to hold the containers, and an outer reel, which holds a spiral. While the containers are being loaded, the outer reel (spiral) is locked to the shell, and the inner reel is rotated. This allows the containers to be indexed along the steps of the inner reel following the path of the spiral. After loading, the two reels are locked together, holding the containers in place. During the processing cycle, the locked reel assembly and containers are rotated at 15–35 revolutions per minute. Since the inner reel is constructed from a drum, the time required for venting of the orbital sterilizer is greatly reduced because of the reduced volume of air to be removed. Steam enters the shell through a slotted trough at the bottom of the shell, and air is exhausted through five 50-mm diameter vent pipes located along the top. The retort drain valve is also open during the first portion of the venting cycle for condensate removal. Generally the venting schedule is completed in 2–3 minutes [14].

36.4.3.5 Continuous Rotary Sterilizers

These systems are composed of at least one heating shell and one cooling shell and are designed for continuous handling of containers at up to 600 cans/min. Containers from the sealing machine enter the first shell through a self-sealing rotary valve which maintains the pressure within the shell. Each shell is designed with a "Spiral T" permanently attached to the wall, and a reel assembly with steps to hold the containers. As the reel turns, the containers follow the path of the spiral through the shell. After a container travels the length of the shell, it either is transferred to another shell by a rotary transfer valve or exits through a discharge valve. The venting and come-up procedures are performed without containers present in the vessel. This allows more flexibility in the design of the operating procedures, compared to batch systems. Continuous rotary sterilizers are fitted with two or three 50-mm diameter vent lines. The typical venting schedule calls for venting the units for 7 min and to at least 105°C with the vent valves wide open and the drain valve at least partially open. Alternatively, the air may be removed through only the bleeders, drain, and purge lines rather than through the main vent lines. While air removal in this manner does take more time than traditional venting methods, it requires less operator involvement [11].

36.4.3.6 Cascading Water Retorts

The Steriflow cascading water retorts are designed and manufactured by Barriquand, Paris, France. The company manufacturers both stationary and end-over-end rotational systems that have been installed internationally for processing foods and pharmaceuticals. Cascading water retorts utilize high-velocity superheated water to sterilize containers of food. Heating and sterilization are achieved by superheated water steaming at a high flow rate over the containers. An overriding air pressure is available for glass jars and flexible and semi-rigid containers, to protect the physical integrity of the container and seal.

A schematic of the retort is shown in Figure 36.4. Water is heated by a welded plate heat exchanger located at the back or at the side of the retort in the middle. On the single door units, the heat exchanger is located at the end opposite the door; on the retorts with the door on both ends, the heat exchanger is located on the side of the shell and water enters at the top center of the retort. The super-heated water is fed into the retort through a distribution manifold. This water is continually recycled through the heat exchanger. In the heat exchanger, steam transfers its latent heat to the internal water, which is then showered from the distribution manifold over the product. The water cascades over the containers, not touching the sides of the shell and only touching a portion of the shell bottom. Therefore, it is not necessary to heat the entire shell during the come-up time, which can save energy. The same water is successively used for sterilizing and cooling the product. Therefore, the cooling is achieved with sterilized water, which means that chlorine doesn't need to be added in cooling water.

After passing over the containers, water passes through filters that keep debris, such as pieces of glass or product, from recirculating through the system. Both the water manifold and the filters are designed to be easily cleaned. The water manifold is a hinged gate at the end or ends next to the door, which can be opened and cleaned regularly. Since the opening for cleaning is opposite the water entrance, all debris is pushed to that portion of the manifold. This design is important to prevent the distribution holes from becoming clogged and perhaps reducing the water flow through a portion of the manifold. The bottom filters are also easy to remove and clean.

The first step is usually a preheat step, or a tempering step in the case of glass jars. Usually a certain minimum time is specified to get a more uniform come-up time for each cook. The second step is an overshoot of both time temperatures. This step is inserted to make sure that the mercury thermometer registers the scheduled temperature at the beginning of the sterilization phase. This step is critical because the controlling probe and the mercury thermometer are not located in the same place; the mercury thermometer lags behind the controlling probe. The third step is the sterilization phase. For this step it is recommended that the operating temperature be 1°C above the schedule retort temperature. The reason, common to most restarting systems, is to make sure that the mercury thermometer is at or above the schedule retort temperature during the entire sterilization phase. Time, temperature, and

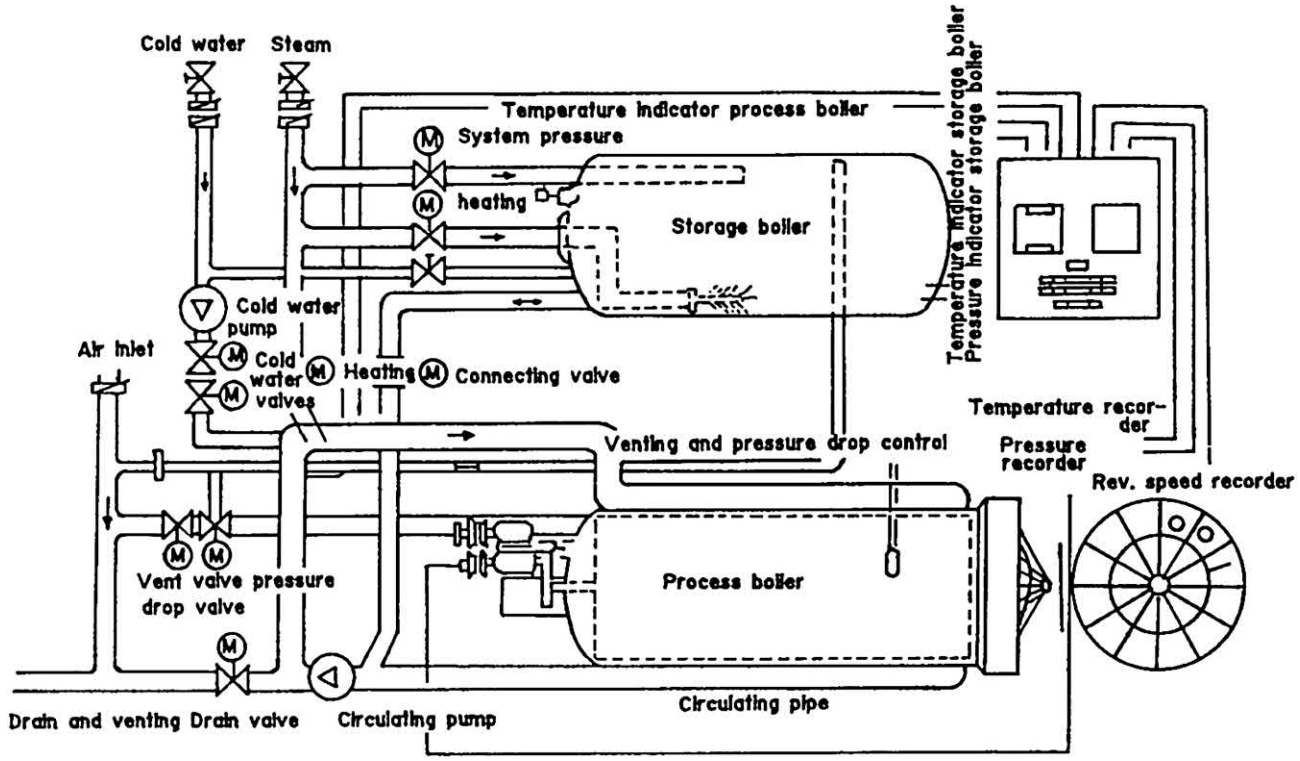

FIGURE 36.4 Horizontal circulating water retort (from Perkins [12]).

pressure are controlled. If the control in temperature drops below the set point, the timer stops until the correct temperature is achieved again. The fourth step is a pressure cooling step to protect plastic or semi-rigid containers from bucking or glass containers from losing their lids during the first few minutes of cooling. The final step is an atmospheric cooling. The actual times, temperatures, and pressure for each program depend on factors such as product formulation, container material and shape, entrapped air, product quality considerations, and steam, water, and air supplies [15].

36.4.3.7 Rotary, Full, Immersion, Hot-Water Sterilizers

The typical rotary hot-water sterilizer consists of two hot-water drums—the upper (storage) drum the lower (working) drum. Sterilization of the food takes place in the storage drum after it receives preheated hot water from the storage drum. During rotation cages turn in the same vertical plane within a rotating framework, called the "rotor insert." Containers in the cages travel in end-over-end fashion in the hot water if loaded in a vertical orientation. Overpressure is usually supplied with steam in the storage drum, although steam or compressed air may be used with a slight installation modification.

As thermal energy in the lower drum is given up to the product, cages, and shell during the process, more energy is introduced into the working drum through an external steam mixing or distribution chamber. Steam is injected directly into the system's circulating hot water from the working drum through a diffuser located within the chamber. There is no direct steam injection into the shell in these units. Water is pulled from bottom or side ports spaced equally along the shell of the working drum, where it is pumped through the mixing chamber for heating. Water travels through top or side water inlet ports into the working drums in a "top-to-bottom" or "side-to-side" circulation pattern. Rotating the cages during come-up, heating, and cooling cycles helps to distribute thermal energy in the system, and reduces the come-up and cool-down cycle times.

Temperature and pressure are independently controlled in these systems. Temperature and pressure sensor connections for the recorder system are located in the thermometer pocket at the horizontal center plane of the working drum. A resistance temperature device (RTD), sometimes referred to as a "PT-100," is located near the right side of the front end of the storage drum and controls the storage drum water temperature. A second RTD controls the temperature in the working drum and is positioned either before or after the mixing chamber in the circulation line or in the mercury-in-glass (MIG) thermometer well. After the heating phase is completed, a portion of the process water is pumped back into the storage drum, where it is heated to the required temperature set point for the next cycle. The circulation pumps are rated at approximately 400 gals/min with a 250-gal/min typical flow rate. The pumps use on/off, proportional valves and valve actuators on the units [16].

36.4.3.8 "Hydrolock" Continuous Cooker/Cooler

Hydrolock is a continuous, agitating cooker/cooler for high-speed-short-time sterilization of a wide variety of sizes and shapes of containers. The system is applicable to the processing of cans, glass jars, semi-rigid plastic and metal containers,

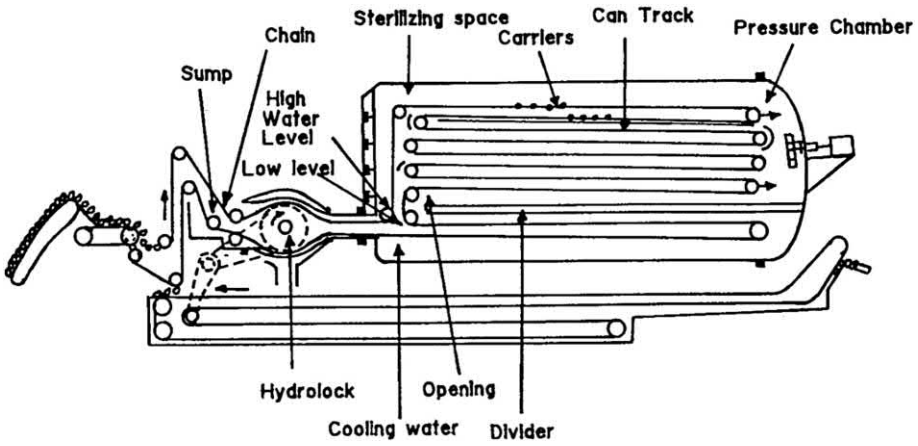

FIGURE 36.5 Hydrolock continuous cooker. (From Brody [20].)

and retortable pouches. It is also capable of processing plastic and metal containers with heat-sealed closures [12, 17–20]. The basic parts of the system are (Figure 36.5) the water lock, cooker/cooler, chain carrier system, cooling system, and water-circulating system. Containers enter and travel through the process between two parallel conveyor chains. These chains enter and leave through water into a rotating pressure lock, sealed partly by water and partly by mechanical means. This facilitates preheating of incoming and precooling of outgoing containers. After loading through the lock, the containers are continuously conveyed through the steam chamber and finally into precooling water in which the conveyor passes. Containers exit through the same rotating pressure lock through which they entered and pass along a cooler conveyor. The hydrolock is equipped to provide overhead pressure during the cooling cycle to the container integrity. Final product cooling is completed in two passes of atmospheric cooling below the pressure vessel. Cans roll in shallow water in a stainless-steel "pan" being pushed by stainless-steel rods attached at their ends to roller chains. Any heating medium can be used with the system: saturated steam, water, or a steam–air mixture. When an over-riding air pressure is required, as with glass containers, aluminum cans, plastic containers, or flexible pouches, air is mixed with the steam by means of one or more turbo fans which produce a homogenous mixture of the two gases.

36.4.3.9 Hydrostatic Pressure Sterilizer

This sterilization method is more commonly known as "hydrostatic sterilization" because the steam pressure in these units is maintained by water pressure. Hydrostatic cookers are continuous pressure cookers in which the operating pressure is maintained by water pressure. The schematic of the cooker is shown in Figure 36.6. Hydrostatic cookers have two components: water chambers and steam chambers. The temperature of steam in the steam chamber is controlled by pressure produced by the water legs and can be regulated by moving the level of water in the leg [13, 19, 21]. Containers are conveyed into the cooker through a water leg at 80°C. This is the down-traveling water leg, and the container temperature begins to increase. As the containers move down this leg, they encounter progressively hotter water. In the lower part of this leg the water temperature reaches about 100°C, and then, near the water seal area next to the steam chamber, the water temperature is about 107°C. In the steam chamber the can is exposed to a temperature of 115 to 130°C, the steam temperature being set to suit the product undergoing sterilization. Upon leaving the steam chamber, the can again goes through a water seal

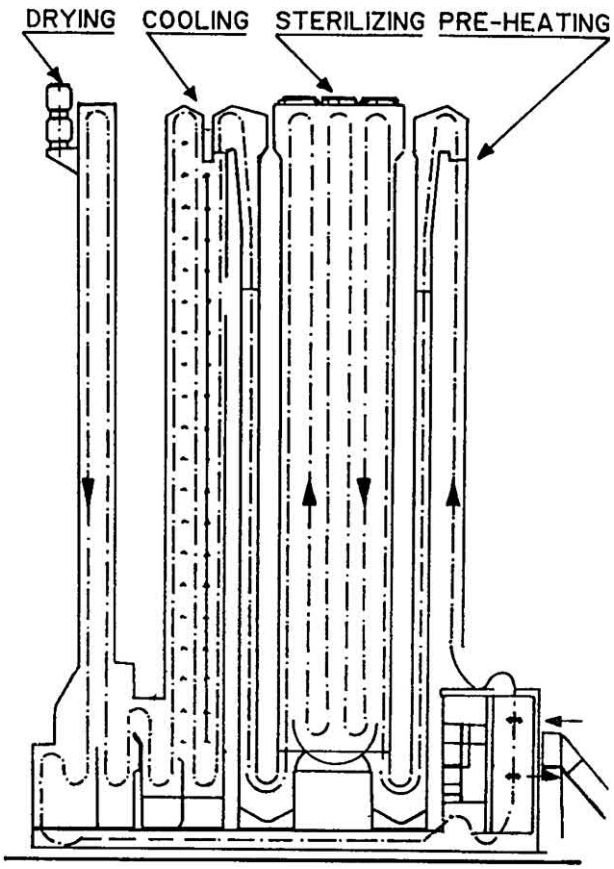

FIGURE 36.6 Hydrostatic pressure sterilizer (internal details). (From Perkins [12].)

into water at a temperature of about 107°C where the cooling cycle commences, under pressure.

36.4.3.10 Hydrostatic Helix

The major advantage of the hydrostatic cooker is its compact size. The hydrostatic cooker has no mechanical valves or locks and thus can be a truly continuous-motion retort. The helical pump or hydrostatic helix consists of a rotating coiled tube in which each turn of the coil is charged at the intake partly with liquid and partly with air. The coil rotates about a horizontal axis. With no pressure at the discharge, the rotating coil may meter liquid at a rate proportional to its rotational speed. With a discharge backpressure, the liquid in each coil turn forms a series of additive hydrostatic legs. The hydrostatic head developed is a function of the number of turns of the helix and the diameter. When the coil is rotated, liquid can enter the coil by gravity flow for one-half turn only, when the first turn (acting as a manometer) is in the upright position. As the coil continues to turn through the next half turn, only air can enter because the "manometer" is inverted. Thus, equal volumes of liquid and gas are alternately introduced into the helix in a repetitive cycle. The helical pump thus operates with many short columns of gas [22].

36.4.3.11 Continuous Pallet Sterilizer

Hydrostatic sterilizers, because of their size and the complexity of their water recirculation systems, are very expensive to construct and erect [12]. The continuous pallet sterilizer is basically a continuous vertical retort through which cans are transported on pallets. The feed and discharge of the pallets are effected, without pressure loss, through venting locks. Each filled, unprocessed pallet load is conveyed by a rack and pinion arrangement into the lock. After the outside pressure door of the infeed lock is closed, steam is introduced, first at atmospheric pressure to purge air from the pallet and chamber and thereafter under pressure to equilibrate the lock with the retort. After the venting-equilibration cycle, the pallet is moved forward until it is at the base of the retort. Pallets slowly ride upward on their four railroad-like wheels. The processed pallets leave the top of the retort through a "let-down" lock. The flexibility of a retort, in terms of container type, is shared by the continuous pallet retort by virtue of the capacity of a round vessel to withstand much higher pressures than a rectangular hydrostatic sterilizer tower. Hot water sprays, overpressured with air and steam with superimposed air pressure may be used as the sterilizing media. This equipment can be used for continuous processing of pouches, semirigid aluminum containers, institutional half steam table trays, and glass jars.

36.4.3.12 Flame Sterilizers

Infrared radiation as an indirect heat source was developed into flame sterilizers/cookers. Flame cookers attempt to increase the temperature differential between the heating source and the food product in order to increase the rate of heat penetration. By increasing the rate of agitation, the probability of burn-on is markedly reduced [13, 20, 22]. Gas burners at 1100°C provide the heat source to impart the high-temperature-short-time effect. The cans are placed in very close proximity (just a few millimeters away) to the burners and are kept in constant rotation, with a temperature differential between the can and contents not exceeding 1°C. Thus, even fully lithographed cans may be heated without damage. There is no possibility of imparting counterpressure, so cans must be fairly rigid to withstand internal steam pressure. With low-viscosity products, extremely high rates of temperature increase (e.g. 0.25°C/s) of contents are possible. The unit depends on continuous axial rotation (about 120 rpm) to move the cans along the burners and to obtain the internal turbulence. Some units have a steam preheat section. A schematic of the unit is shown in Figure 36.7 [23]. The steriflame units consist of three sections. The first one is a steam preheater, where the cans are heated to a temperature of approximately 100°C. In the second section, the cans roll through a series of open flames at 1100°C produced by specially constructed gas burners. The rolling motion of the cans increases the rate of heat transfer into the whole mass of the food. Next the cans pass through an intermittently heated burner holding section for about 4 to 5 minutes. Spray cooling follows the heating cycle. The total elapsed time in the cooker is generally less than that required for batch retorting.

36.4.3.13 Fluidized-Bed Sterilization

The fluidized-bed retort is a cooker in which sand or ceramic pellets are used as the heat transfer medium. The medium is

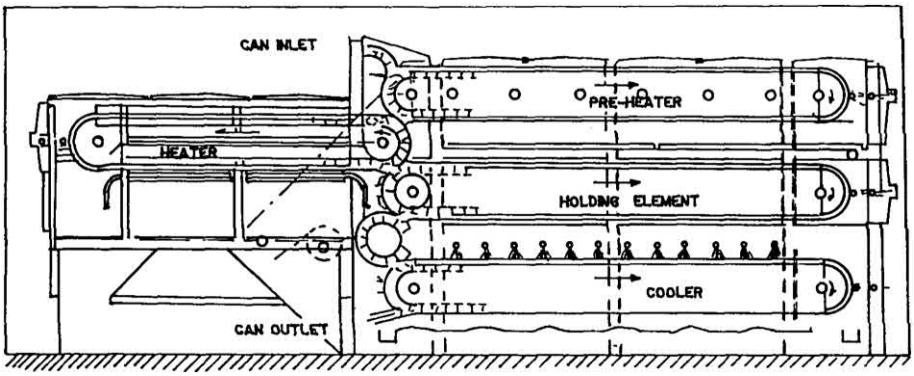

FIGURE 36.7 Flame sterilizer (longitudinal section). (From Beauvois et al. [23].)

kept hot and fluid by a flame underneath and an air stream. The particles behave much like boiling liquid. Cans move through the bed, meeting the same resistance as they would if the medium were a thick liquid receiving some abrasive effect from the particles [20]. The main advantage of the system is the close temperature control (e.g., 1°C at 1000°C) and that it does not require a pressure chamber. The process is continuous, and several sizes may be sterilized simultaneously. The main disadvantages are the possibility of burning and discoloration of the can surface, and damage of the can seals.

36.4.3.14 Hot Sterilization

Hot air with very high velocity (approximately 600 m/min) is employed to decrease the thickness of the non-turbulent air layer adjacent to the can surface. High-velocity air in excess of 150°C also creates a large temperature differential between the surface and the contents. Cans are axially rolled through to create forced convection within the can contents, thus reducing the possibility of burning or overcooking [13].

36.4.4 Temperature Distribution in the Retort Systems

There are numerous critical tests that must be performed to properly establish commercial sterility processes for any retorting application. The establishment of the "scheduled" (i.e., commercial sterility) process by a process authority depends not only on the ability of the food processor to properly control critical factors which relate to the pre-retort preparation of the product and the package in which the food is to be sterilized but also on those factors which specifically relate to the delivery of the thermal process to the packaged food. The manner in which the sterilizer is designed, installed, and operated is of critical importance. Failure to address these concerns will have a direct and significant impact upon the lethality (sterilization value) designed into (and actually obtained in) the process. The FDA not only requires that temperature distribution tests be performed but also expects that certain data be collected and procedures followed. In addition, FDA [21CFR113.4 (b) (10) (i)] and USDA [9 CFR 318(381). 305(C) (2)(x)] regulations require that these data be evaluated by a "recognized competent process authority."

The traditional means by which acceptable process delivery conditions are verified is to use thermocouples, RTDs, or thermostats to generate time/temperature histories at pre-assigned strategic locations inside the sterilizer. These data are collected from "zero time" (when the heating medium first contacts the containers in the working drum) until a "to-be-determined time" at which all temperature-measuring devices attached to the sterilizers indicate same in the recording devices. Most important, the time at which the lowest temperature lead meets the true "set point" temperature of the control program is critical. This exact time/temperature condition is the traditional process calculation reference point from which process hold times are determined. The "official reference instrument" against which all other sterilizer temperature control devices are adjusted is the MIG thermometer. All thermocouple lead readings must be able to compare to the MIG thermometer readings. Temperature distribution is, then, the uniformity of sterilizer temperatures and the stability of sterilizer temperatures at any given time during the entire process cycle, including the come up, holding, and cooling phases.

Temperature distribution in batch, rotary, or hot-water sterilizers is affected by numerous critical plant conditions and system future: (i) *Product initial temperature*: Lower initial temperatures usually lengthen retort come-up time and worsen temperature uniformity and stability. (ii) *Storage drum temperature*: The storage drum temperature is usually programmed to be 15–20°C above the targeted control set point in the working drum. If the hot water drop temperature is too high, some semi-rigid and flexible containers may be damaged and the temperature gradients between outside and inside the container cage position can be excessive. If the hot-water drop temperature is too low, too much heating in the working drum must then occur, also creating wide temperature gradients. (iii) *Working retort venting*: Venting the working retort for too long in sterilization removes excessive energy from the system, extends come-up time, and forces more mixing chamber steam injection during the come-up phase. Too short a venting time does not allow the storage drum/working drum fill step to occur properly and causes pressure control problems in the working retort. Ideally, a venting time of approximately ½ min/cage is appropriate (i.e., 2 min for a four-cage 1,100-mm unit). (iv) *Working retort RTD location*: The control RTD may be located before or after the mixing chamber in the water circulation line. When the RTD is located after the mixing chamber, the control device measures the hottest water in the circulation system; this results in a less aggressive steam valve response and a slower but smoother ramp to temperature in the come-up phase. If the RTD is located before the mixing chamber, the control device measures the coldest water in the system, and the steam valve to the mixing chamber experiences a "response lag" by fully opening for too long. This causes temperature overshoot at the end of the come-up period. (v) *Container type and geometry*: Low-profile containers (cans, pouches, plastic trays, or bowls) must be filled into racking systems, which may create a considerable number of cage layers. Increased layers create increased resistance to the heating medium flow. Generally, full loads of large cylindrical containers exhibit a shorter come up time and improved temperature uniformity in all process phases. (vi) *Container handling system*: The cages, dividers, and racks for handling the containers must be designed to provide maximum cross-sectional open area (CSA) created by slots or perforations in the sides and bottoms of the cages. Spacer mats or divider sheets between layers of containers must be designed for maximum open area to enhance water flow past container surfaces. (vii) *Rotational speed*: Generally, as rotational speed increases come-up time decreases and temperature uniformity and stability improve. (viii) *Number of cages*: The larger the unit and the greater the number of cages, the slower the come-up time, and the greater temperature differences throughout the drum must be overcome. (ix) *Retort design and operating environment*: Regardless of the retort

manufacturer, it is advisable to perform temperature distribution studies in each batch, full-immersion, rotary hot-water retort. Design modifications, valve settings, blocked ports, and the number of retorts operating simultaneously in conjunction with other steam demands in the plant will affect test results and should be monitored closely. Rotary retards to be added to an existing line must also be tested for temperature distribution [16].

36.4.5 Exhausting

The exhausting of containers for the removal of air should be controlled so as to meet the conditions for which the process was designed. Vacuum in canned foods may be obtained by pre-heating foods prior to closing. In producing a vacuum by this means, the product may be heated prior to filing, or it may be heated both before and after filling. Heat in this case is employed to expand the product, to expand and drive out the occluded and dissolved gases in the product, and to reduce the air in the headspace before closure. The length of heating and the final temperature attained before closure have a very important relationship to the ultimate vacuum in the can. Heating may be accomplished by passing the filled can through a steam or hot water exhaust box. It is common to refer to exhaust box treatment as "thermal exhaust" and to preheating before filling as "hot fill." Exhaust boxes are generally best adapted for canned foods that can readily be heated, such as brine- and syrup-packed fruits and vegetables. The major disadvantages of exhaust boxes are in their bulkiness and their large steam requirements. In mechanical vacuum closure by high-speed vacuum closing machines the filled cans while cold or at a rather low temperature are passed into a clincher which loosely clinches the covers without forming an air-tight seal. The cans are then transferred through a suitable valve into a vacuum chamber, subjected to vacuum for an instant while in the vacuum chamber, sealed, and then ejected through another valve. Vacuums drawn on the machine while the cans are in the vacuum chamber may be varied over a wide range, depending mainly on the desired final vacuum in the can and also on the temperature of the liquid contents. This method of exhausting air from canned foods subjects the contents to a vacuum for a rather short interval of time before closure. Therefore, the air is withdrawn mainly from the headspace and only partially from the product itself and proper adjustment of the headspace is necessary for proper performance.

36.4.6 Quality of Canned Foods

36.4.6.1 Plant-Origin Foods

The purpose of heat sterilization is to extend the shelf life of foods while minimizing the changes in nutritive value and eating quality. Differences between the heating characteristics of microorganisms, enzymes, and sensory or nutritional components of foods are exploited to optimize processes for the retention of nutritional and sensory qualities. This is achieved in practice by a reduction in size or cross-sectional area of containers, by agitation during processing, or by aseptic processing. The extent of thermal processing which a food receives is dependent upon the composition and physical characteristics of the product and is the result of a combination of time and temperature. Physicochemical changes occurring during processing and storage are the factors which determine the product quality in terms of both its sensory properties and its provision of nutrients to the consumer. Reactions take place during both the process itself and in subsequent storage. Generally the changes which occur during storage are slow, particularly when compared with those occurring in an equivalent unprocessed material, and it is on this basis that heat preservation is effective in providing materials outside their normal seasons and in a convenient, prepared, often formulated, form ready for consumption or reheating and then consumption. The physical and chemical reactions which occur during processing can be desirable or undesirable, are often more significant and certainly occur much more rapidly than those during storage. The degree of heat processing varies according to the product. In turn the changes occurring in processing are influenced by the time and temperature of the process and the composition and properties of the food material [24] and its environment [25].

36.4.6.1.1 Sensory Quality

The heat process itself has a major effect on the quality of a food product and is responsible for a range of changes taking place. Starch gelatinization and structural protein denaturations have a direct influence on the texture of a food product. Heat-induced reactions such as the Maillard reaction affect the color and flavor as well as the nutritional status of the food [26, 27]. In general, changes that occur before the heat process are less important than those during or after processing since it is the manipulative and thermal procedures of food production which have the greatest effect on tissue damage and the resultant mixing of cell contents from different materials.

36.4.6.1.2 Texture

The tissue damage which occurs during the heat processing of plant material is of two types. These are destruction or damage to the semi-permeable cell membranes, and disruption of the intercellular structures with resultant cell separation [28]. The effects of these types of tissue damage are a loss in cell turgor and cellular adhesion which give rise to loss of crispness and softening of the heat-processed product. Other major influences on the texture of heated foods arise from the denaturation of proteins. Even with relatively mild heating conformational change affecting the tertiary structure of protein can be observed [29]. Denaturation of the proteins may follow. The hydrogen bonds, maintaining the secondary and higher structure of protein, rupture, and predominantly random coil configuration occurs [30]. This leads to considerable changes in the chemical and physical properties of proteins due to losses in solubility, elasticity, and flexibility [29, 31]. This mechanism also causes enzyme inactivation and

breakdown of proteinaceous toxins and antinutrients. They cause turbidity leading to either a precipitate or gel, which will greatly alter their water-holding capacity and also lead to increased thermal stability [32]. In fruits and vegetables, softening is caused by hydrolysis of pectic materials, gelatinization of starches, and partial solubilization of hemicelluloses, combined with a loss of cell turgor. Calcium salts may be added to blanching water or to brine or syrup, to form insoluble calcium pectate and thus to increase the firmness of the canned product. Different salts are needed for different types of fruit (for example calcium hydroxide for cherries, calcium chloride for tomatoes, and calcium lactate for apples) owing to differences in the proportion of demethylated pectin in each product.

36.4.6.1.3 Color

The color of a food product is determined by the state and stability of any natural or added pigments and the development of any coloration during processing and storage. Natural pigments are generally unstable compounds that are broken down on heating but whose stability is dependent upon many factors. In fruits and vegetables, chlorophyll is converted to pheophytin, carotenoids are isomerized from 5,6-epoxides to less intensely colored 5,8-epoxides, and anthocyanins are degraded to brown pigments. Anthocyanins are fairly heat-stable compounds but take part in a wide range of reactions, e.g., with ascorbic acid, sugar breakdown products, such as hydroxymethyl furfural, and other reactive phenolics which bring about their breakdown [33]. Factors that accelerate degradation include high levels of oxygen in the product and storage temperature. Conversely, anthocyanins can be undesirable in a product and can be produced on thermal treatment of leucoanthocyanidin [34, 35]. They give rise to defects, such as very dark broad beans and red gooseberries. Other problems can occur with anthocyanin pigments due to the formation of metal complexes, for example, the bluing of red fruits and the pinking of pears when exposed to tin [36, 37]. The flavonoid rutin, present in asparagus, can also form a complex with iron causing dark discoloration in lacquered cans where iron dissolution can occur [38] and in which the colorless tin complex is not formed.

Carotenoids are mostly fat-soluble and are responsible for yellow, orange, and red coloration. They are unsaturated compounds and are therefore susceptible to oxidation, giving rise to off-flavor and bleaching. In addition, two types of isomerization can occur, namely, *cis–trans* isomerization and epoxide isomerization, which can give rise to lightening of the color. The temperature of storage is considered to have a greater effect on the isomerization than the heat process itself. The two major groups of porphyrin-based pigments are chlorophyll and the heme compounds, both of which are very sensitive to heat. On processing, chlorophyll is converted to pheophytin with an associated loss of green color [38]. Several approaches have been taken to try to reduce the color loss such as adjusting the pH [39, 40] and the use of HTST treatments. In the latter case although improvements were observed after processing these were lost during storage [41].

Betalins are water-soluble pigments, which are susceptible to oxidation and loss of red color. Browning of heat-preserved beetroot products is an example where residual oxygen in the product or headspace causes the appearance of a chocolate-brown color. Heat processing itself in the presence of oxygen has a major effect on the end-product quality, and this is demonstrated by the comparison of products packed in plain tin-plate cans with the identical material processed in lacquered cans or glass jars. In the plain tin-plate container dissolution of the tin during processing removes a major proportion of oxygen from the pack, and little is available to react with the food. Some products such as pale fruits, tomatoes and tomato formulations, mushrooms, and milk products are particularly susceptible to such heat-induced oxidative changes. It has been demonstrated that a brownish color develops in beans dipped in tomato sauce packed in different container types [42]. Ascorbic acid is often used as an antioxidant and can be effective in improving color in certain products, e.g., mushrooms. It can be degraded to produce reactive compounds, which further react to form brown pigments.

36.4.6.1.4 Flavor

Generally, heat preservation does not significantly alter the basic flavors of sweetness, bitterness, acid, or salt. In fruits and vegetables, changes are due to complex reactions which involve the degradation, recombination, and volatilization of aldehydes, ketones, sugars, lactones, amino acids, and organic acids. Major changes can occur in the volatile flavor components. One of the most important sources of volatiles is lipid oxidation or oxidative rancidity. The three stages are (i) initiations, (ii) propagation (i.e., formation of highly reactive hydroperoxides), and (iii) termination. The initiation uptake of oxygen, in the presence of catalysts, such as metal ions or metalloproteins can initiate oxidation, but it can also be enhanced by heat or light. The reaction does, however, have low activation energy (4–5 kcal $mole^{-1}$). The hydroperoxides formed take part in secondary reactions to give rise to a range of volatiles including aldehydes, ketones, and alcohols, and they produce typical rancid or stale off-flavors.

Volatile flavor compounds are also produced via the Maillard reaction. Since the first scheme for the reaction was put forward [43] a great deal of research has been undertaken. The reaction occurs during heating and extended storage, is influenced by water activity, with an optimum for flavor generation at intermediate values of around 30% water [44], and is accelerated by high pH and buffers such as phosphates and citrates [45]. The first stage of the reaction is fairly well-defined and involves the condensation between carbonyl groups of the reducing carbohydrates and the free amino acids or protein and rearrangement to produce amatory compounds. This leads to loss of protein nutritional quality but does not affect the sensory properties significantly [46]. The second stage is very complex and gives rise to numerous products, many volatiles, and is responsible for many characteristic flavors and off-flavors in food materials. The loss of volatile constituents can also present problems in heat-preserved foods. The breakdown of essential oils in citrus products can result

from oxidation. Packaging can also have a direct influence on volatile scalping.

36.4.6.1.5 Nutrients

Both physical and chemical reactions occur in heat-preserved foods, which influence nutritive value (Table 36.1). Physical factors such as the loss of soluble nutrients, or leaching, can be significant for products in which there is a carrying liquid discarded before consumption. Chemical reactions include heat damage to labile nutrients such as vitamins. One of the most fundamental changes which can occur in a heat-preserved product is the movement of water and solids within the food material during processing, storage, and reheating. In a formulated product or a product in which the entire pack contents are consumed, such changes can be largely disregarded, from the nutritional point of view, in that they do not alter the total amount of the nutrients consumed. Products which are packed in a liquor which is discarded before consumption often exhibit dilution, dehydration, or loss of total solid materials from the edible portion. Sterilized soya-meat products may show an increase in nutritional value owing to an unidentified factor that decreases the stability of the trypsin inhibitor in soybeans.

36.4.6.1.5.1 Proteins Heat preservation can lead to both desirable and undesirable changes in the nutritive quality of proteins. They are susceptible not only to heat but also to oxidation, alkaline environment, and to reaction with other food constituents such as reducing sugars and lipid-oxidative products. The total amount of crude protein generally appears relatively unchanged due to heat processing [47, 48] but can suffer from leaching into the liquid component of some products [49]. The crude protein levels, however, appear to be stable during subsequent storage of canned vegetables [47, 48]. The changes that occur are associated with tertiary structure, functionality, chemical changes related to digestibility, and amino acid availability. Canning of potatoes also leads to losses of amino acids though this has been shown to vary depending on the specific gravity of the potato [50]. Lysine is again particularly vulnerable with a reduction in its availability of about 40%. Some of the losses found in canned potatoes may be due to the leaching of the protein into the brine [49] although the major cause of loss of amino acids on heat preservation is the Maillard reaction. Soybeans and many other legumes also undergo improved protein digestibility and bioavailability, especially of the sulfur-containing amino acids on heating due to inactivation of trypsin inhibitors and unfolding of the major seed globulins.

TABLE 36.1
Effect of Heat Processing on Major Nutritional Components

Nutrient	Effect
Dry matter	Loss of total solids into canning liquor
	Dilution
	Dehydration
Protein	Enzymic inactivation
	Loss of certain essential amino acids
	Loss of digestibility
	Improved digestibility
Carbohydrate	Starch gelatinization and increased digestibility
	No apparent change in content of carbohydrate
Dietary fiber	Generally no loss of physiological value
lipids	Conversion of *cis* fatty acids to *trans* by oxidation
	Loss of essential fatty acid activity
Water-soluble vitamins	Large losses of vitamins C and B, due to leaching and heat degradation
	Increased bioavailability of biotin and niacin due to enzyme inactivation
Fat-soluble vitamins	Mainly heat stable
	Losses due to oxidation of lipids
	Losses due to leaching
Minerals	Possible increase in sodium and calcium levels by uptake from canning liquor

36.4.6.1.5.2 Vitamins The effect of heat preservation on vitamins is generally detrimental although mild heating conditions can have beneficial effects on the bioavailability of certain vitamins, particularly biotin and niacin. This is due to enzyme inactivation and the inactivation of binding agents [51]. The stability of vitamins varies under different conditions with vitamin C and thiamin being most susceptible to degradation through heating. The fat-soluble vitamins are the more stable of the two sets, although these can be degraded by oxidation especially when heated. Losses of water-soluble vitamins during processing can be considerably higher. Vitamin C is the most labile of the vitamins and can be lost during storage of the fresh material, food preparation, washing, and blanching as well as by degradation on heating and leaching into a carrying liquid during the process. Studies on garden peas and carrots have shown that as much vitamin C can be lost in the storage of the fresh produce for 7 days prior to cooking as that lost in canning. Much of the vitamin C lost during canning is leached into the canning liquor. Thiamin is the most heat sensitive of the B vitamins especially under alkaline conditions, and it is also susceptible to leaching during any washing or blanching stages. Thiamin, however, is less labile than vitamin C and retention of 60 to 90% is usual in canning [52]. Folic acid and pyridoxine are also susceptible to degradation by heating and, in the case of folic acid, also by oxidation. Canning of potatoes can lead to losses of vitamins up to 30% [53]. Riboflavin and niacin are both relatively stable on heat preservation although riboflavin is very sensitive to light and will undergo degradation in the presence of both heat and light together [54]. Heat-preserved foods often require less cooking than fresh foods, and the differences in the vitamin content between the fresh and the processed food at the point of consumption can often be negligible. In canned fruits and vegetables, significant losses may occur in all water-soluble vitamins, particularly ascorbic acid/vitamin C.

36.4.6.1.5.3 Minerals Minerals are generally stable in most of the conditions encountered in heat preservations i.e.,

heat, air, oxygen, acid, or alkaline. Losses of minerals, however, can occur during processing, especially of vegetables, due to leaching into canning liquor. Conversely, certain minerals, for instance sodium and calcium, can be taken up by the food from the cooking or canning liquids. Comparisons between fresh and canned vegetables have shown higher ash content in canned products in all cases. This is due to the uptake of sodium, and to a lesser extent of calcium from the brine. Between 15 and 50% of potassium can be lost primarily by leaching in the canning of vegetables. Slight leaching of zinc and negligible changes in iron content occur during processing. Heating has been seen to increase the bioavailability of iron in spinach, and the presence of fructose also leads to increased iron bioavailability [55]

36.4.6.1.5.4 Carbohydrates Carbohydrates are less susceptible than most other food compounds to chemical changes during heat preservation. The levels of total and available carbohydrates in vegetables have been found to be very stable on canning and subsequent storage of the canned vegetables. However, there are some effects of heat on various carbohydrates. The effect of sugar on protein and iron bioavailability and the relationship between starch, texture, and palatability are more important. Gelatinization of the starch also aids digestibility of foods. A good example of this [53, 56] is the potato which in its raw state is largely indigestible. The exact effect of heat preservation on various types and constituents of dietary fiber has not been fully investigated. Cellulose, the main constituent of dietary fiber, hemicelluloses, and pectins are together responsible for the structure and texture in plant foods [57, 58] and can be disrupted by heating which leads to a softening of the food and increased palatability as discussed earlier, generally, without any loss in the physiological value of the dietary fiber. Over-heating can lead to breakdown in the cells enabling water-soluble nutrients, for instance certain minerals, vitamins, and pectins, to be leached out. Although dietary fiber is considered to be largely unaffected by heat processing, the exact relationship between time/temperature conditions, dietary fiber breakdown, and the extent of nutrient loss due to fiber breakdown requires further study.

36.4.6.1.5.5 Lipids Lipids, especially unsaturated lipids, are prone to oxidation when heated in the presence of air or oxygen, resulting in losses in nutritional value of the food product. Although the major effect of lipid oxidation is in the flavors of foods, oxidation can lead to a conversion of the natural *cis* fatty acids to *trans* fatty acids [55]. The digestion and absorption of *trans* fatty acids are comparable to that of the *cis* fatty acids, and their nutritional value as an energy source is not affected. However, *trans* fatty acids do not generally possess essential fatty acid activity, i.e., as precursors of prostuglandins, thromboxanes. This activity is dependent on a *cis* 9, *cis* 12 methylene interrupted double bond system, but provided that sufficient linoleic acid is consumed the *trans* fatty acids do not appear to inhibit essential fatty acid metabolism [59, 60]. The oxidation of lipids has also been implicated, as previously noted, in the loss of protein quality and can inhibit the activity of the fat-soluble vitamins A, D, and E as well as vitamin C and foliate. The oxidation of fats in processed foods, however, can be controlled by the exclusion or minimization of oxygen and the use of antioxidants. The effects of heat preservation on the nutritional value of fats can therefore generally be considered as negligible.

36.4.6.2 Animal-Origin Foods

36.4.6.2.1 Color

The time–temperature combinations used in canning have a substantial effect on most naturally occurring pigments in meat foods. The red oxymyoglobin pigment is converted to brown metmyoglobin, and purplish myoglobin is converted to red-brown myohaemichromogen. Maillard browning and caramelization also contribute to the color of sterilized meats. However, this is an acceptable change in cooked meats. Sodium nitrite and sodium nitrate are added to some meat products to reduce the risk of growth of *Clostridium botulinum*. The resulting red-pink coloration is due to nitric oxide myoglobin and metmyoglobin nitrite. Loss of color is often corrected using permitted synthetic colors.

36.4.6.2.2 Flavor and Aroma

In canned meats, there are complex changes (for example pyrolysis, deamination, and decarboxylation of amino acids, degradation, Maillard reactions and caramelization of carbohydrates to furfural and hydroxymethylfurfural, and oxidation and decarboxylation of lipids). Interactions between these components produce more than 600 flavor compounds in ten chemical classes [61, 62]. Other volatiles have been identified as having a significant effect on the flavor of foods and perhaps one of the most dramatic is the development of "catty taint." This is an extremely unpleasant and potent odor produced by the reaction of unsaturated ketones, notably mesityl oxide, with natural sulfur-containing components of the food [61, 62]. Heating is essential in the formation of the taint, and incidents have been widespread due to the diverse availability of the unsaturated ketones. Examples include processed meat products using meat from cold store, painted with a material containing mesityl oxide as a solvent contaminant [63], canned ox tongues which had been hung on hooks coated with a protective oil [64], and pork packed in cans with a side seam lacquer which had been dissolved in impure solvent [64, 65].

36.4.6.2.3 Texture

In canned meats, changes in texture are caused by coagulation and a loss of water-holding capacity of proteins, which produce shrinkage and stiffening of muscle tissues. Softening is caused by hydrolysis of collagen, solubilization of the resulting gelatin, and melting and dispersion of fats through the product. Polyphosphates are added to some products to bind water. This increases the tenderness of the product and reduces shrinkage. Small changes in the viscosity of milk are caused by modification of K-casein, leading to an increased sensitivity to calcium precipitation and coagulation.

36.4.6.2.4 Nutrients

Canning causes the hydrolysis of carbohydrates and lipids, but these nutrients remain available, and the nutritive value of the food is not affected. Proteins are coagulated, and, in canned meats, losses of amino acids are 10–20%. Reductions in lysine content are proportional to the severity of heating but rarely exceed 25%. The loss of tryptophan and, to a lesser extent, methionine reduces the biological value of the proteins by 6–9%. Vitamin losses are mostly confined to thiamin (50–75%) and pantothenic acid (20–35%). However, there are large variations owing to differences in the types of food, the presence of residual oxygen in the container, and methods of preparation (peeling and slicing) or blanching. In some foods, vitamins are transferred into the brine or syrup, which is also consumed. There is thus a smaller nutritional loss. Heat sterilization of meat leads to a reduction in digestibility of the meat proteins and damages amino acids especially the essential sulfur-containing amino acids and lysine, with 10–15% losses in beef [66]. Heat preservation has two important effects on the quality of foods: (i) Many of the changes (sensory or nutritional) which occur during the thermal process are not restricted to heat-preserved foods. In many instances the process replaces the conventional cooking which the food receives prior to consumption. Reheating the heat-preserved food is a relatively mild treatment that does not significantly affect the quality. (ii) Heat-preserved foods make available to the consumer a wider choice of sensory experiences and nutritional benefits without the constraint of seasonality and the burden of preparation.

36.4.7 Packaging of Canned Foods

Under the regulations, "hermetically sealed container" means a container that is designed and intended to be secure against the entry of microorganisms and to maintain the commercial sterility of its contents after processing. The container is an essential factor in the preservation of foods by canning. After canned foods are sterilized, it is the container that protects the canned food from spoilage by recontamination with microorganisms. It is then most important for the success of the canning operation to use good-quality, reliable containers and properly adjusted closing machines. Thus, the seams and closures produced will be according to the guidelines necessary to prevent access of microorganisms into the container during the cooling operation and during the shelf life of the product.

36.4.7.1 Tin-Plate Cans

Today the choice is from among (i) tin-plate body and ends, (ii) tin-plate body and one end, aluminum convenience end, (iii) three-piece aluminum can (rare, but available and used, with adhesive side seam, for alcoholic cocktails), (iv) tin-free steel with tin end, tin-free steel end, aluminum end, or a combination, (v) tin-free steel body, (vi) adhesive joined side seam, (vii) welded side seam, (viii) draw and iron two-piece aluminum can, (ix) conventional top chime, (x) neck-in top flange so that chime is flush with body, and (xi) draw and iron two-piece steel cans not commercially available except in small sizes for aerosol cans [20].

36.4.7.1.1 Two-Piece Cans

All of the major and secondary can-making and can-handling equipment manufacturing firms produce two-piece draw and iron cans. One of the significant commercial cans is a very small 28 to 57 g (1 to 2 oz.) unit. It is evident that a two-piece steel can would eliminate the long seam and one double seam, and thus preclude two sources of potential leakage. The amount of metal used would be reduced below that used for a three-piece. Two-piece steel cans offer the advantages of a two-piece aluminum can at a lower price [20].

36.4.7.1.2 Three-Piece Cans

Three-piece "sanitary cans," consisting of a can body and two end pieces, are used to seal heat-sterilized foods hermetically and also for other food products like powders, syrups, and cooking oils. Presently, the three-piece cans are widely used, but other cans like two-piece cans, aluminum cans, and other flexible containers are slowly replacing them [20].

36.4.7.1.3 Aluminum Cans

The main applications are where inherent advantage can be realized over the tin-plate such as lower shipping expense, freedom from food and can black sulfide discoloration or rust, easier puncture opening, and where special easy-opening features are desirable [20]. Steel cans are so well-established in the canning industry that exceptionally good reasons are required before a change of material is contemplated. The future use of aluminum for cans, for processed food use, to a great extent depends on the price at which it may be sold to the users, relative to that of an equivalent steel can. Aluminum cans offer advantages of product quality, and economy for the canning of certain food products. The use of easy-open lids is also a significant point which has a strong appeal. Aluminum cans do not rust, and their appearance, always bright, can be an important sales argument. An important advantage of aluminum cans is that they are lead-free. But aluminum cans dent easily, abrade, and are not interchangeable with steel cans.

36.4.7.1.4 Collapsible Tubes

Aluminum may also be used in the form of collapsible tubes for packaging processed food products. Sterilized foods packaged in collapsible tubes for the feeding of astronauts and high-altitude aviators have been developed. The aluminum tube fitted with a hollow handled plastic spoon, which can be attached to the neck of the tube, should make a desirable and convenient package for feeding infants or bedridden patients [20].

36.4.7.1.5 Composite Cans

Another development is the foil/fiber can, more commonly called the composite can. Used earlier for refrigerated biscuit dough, this material is now being used for frozen concentrated orange juice. The composite spiral wound can of fiber/polyethylene/aluminum foil has the major share of the juice and

juice drink frozen concentrate canning. Composite cans have been successfully employed for shortening and with polyvinylidene chloride coating, for vacuum packing of roasted and ground coffee. There has been considerable publicity on the use of composites, for beer, hot fills, pasteurized, and even retorted foods [20].

36.4.7.2 Glass Containers and Metal Closures

36.4.7.2.1 Containers

The chemical and physical properties of glass make it an ideal container material for canned foods. It is a chemically stable material. However, in long-term storage of aqueous solutions, and most particularly of acid foods, a very small amount of alkali may extract from the glass, and in some instances, lesser amounts of SiO_2 or silica. These materials are commonly found in all food products; therefore, the glass container is considered quite inert and is nonadditive in the packaging of most, if not all, food products. It does not support or facilitate microbial growth on its surface, and like metal it is impermeable to gases, liquids, bacteria, and odors. One very apparent characteristic of the glass container is its transparency. While the visibility of the product contained is attractive to the consumer, it does impose restrictions on the canner as to the appearance of the product [67]. Commercial glass jars are formulated and designed to withstand the thermal shocks normally encountered in the canning process. The maximum temperature shock as measured by the temperature differential is generally 45°C. However, they can withstand wider temperature differentials, but only under certain conditions. They are also designed to resist the mechanical shocks normally encountered in a well-designed and maintained filling and packaging line. Their resistance to vertical pressure allows the application of various capping methods and stacking [67].

36.4.7.2.2 Metal Closures (Caps)

The various metal closures that are used in food canning are (i) Twist-off or Eurotwist, (ii) Eurocap and EurocapX, (iii) Pry-off, (iv) Press-twist (PT), and (v) Deep-Press (DP) [67]. The metal closure along with the sealant is designed specifically for each type of glass finish to permit the attachment of a proper seal and efficient closure. For shipping and storage, the nonstackable caps are packed in bulk in cartons, with or without plastic liners, and the stackable caps in overwrapped rolls. The cartons are palletized and either shrouded or strapped. Each carton should be labeled to identify the contents and manufacturing lot. Staples should not be used to close the cartons because they may contaminate the closures [67].

36.4.7.3 Retortable Pouches

Thermally processed laminate structures are made out as retortable pouches. Shelf life, toughness, resistance to puncture, and ability to withstand high temperatures are some of the important characteristics for selecting materials for flexible containers. The retort pouch was designed to be a package that would offer the shelf-stability of canned foods with the quality of frozen foods. The materials configuration of this package has been enhanced over the past several years to bring the pouch even closer to this goal. Typically, the retort pouch consists of a 0.5-mm polyester film laminated to 0.000035- or 0.0007-in-gauge aluminum foil. This is in turn laminated to a 3-mm modified polypropylene film. Each of these three substrates plays an important role in the finished package [68]. On the outside, the polyester provides toughness, abuse-resistance, and printability. The package can be printed with color ranging from simple one- and two-color instructions to full-color vignettes of the food product. The actual printing is applied to the "reverse" side of the polyester film, trapping the inks between laminates to protect against scuffing. In the middle, the aluminum foil is the key to the retort pouch's being a completely shelf-stable food package, with no expensive freezing or refrigeration required. Aluminum is the lowest cost barrier to light, moisture, oxygen, and microorganisms. On the inside, the polypropylene film performs two important functions; first, it is inert and does not react to food, so that virtually the entire range of processed foods can be packaged in this one basic material. Second, it provides exceptionally strong heat-seals that can withstand the pressure and temperature demands of retorting and contribute to a shelf life at least equal to that of cans [68].

36.4.7.3.1 Advantages of the Pouch

The retort pouch is an integral component of the food distribution system with food product quality and package convenience. The thin profile and increased surface area of the retort pouch permit rapid heat penetration and much more efficient processing than with cans. Typical time savings in the cook cycle of a retort process are up to 40%. This reduction in heat exposure results in improved food product quality—better taste, color, and texture than similar products processed in cans. There is also a potential for nutritional advantage as well, particularly where heat-sensitive nutrients are concerned. Packing a food in a retort pouch results in a better-tasting product [68].

36.4.7.3.2 Advantages for the Consumer

From the consumer's viewpoint, the retort pouch is certainly the most convenient food package. Completely shelf-stable, retort-pouched foods may be stored in the cupboard along with other dry goods. Foods packed in retort pouches are sterilized and are ready to eat. Foods may be heated to serving temperatures before consumption. This can easily be accomplished by heating in boiling water for about 5 minutes. In this manner a variety of foods may be conveniently prepared at the same time, with no messy pots and pans to scrub. With the advent of the microwave oven, the true convenience of food preparation in boiling water is now less utilized. All that boiling water does to a retort-pouched food product is heat it; since the temperature of the boiling water is reasonably constant, the pouch can remain in the pot for 6 to 8 minutes and still deliver a satisfactory result, while the consumer is occupied elsewhere. In addition, retort pouched foods are easily prepared in microwave ovens. The contents are poured onto the serving plate and heated for about 1–2 minutes. The added

stiffness of the aluminum foil makes the retort pouch easy to tear open, using the notches provided. Disposal of empty pouches after use is extremely convenient, as they are easily flattened and contain no dangerous sharp edges. This is particularly important in foodservice operations, where more pouches are disposed of and take more space [68].

36.4.7.3.3 Advantages to the Processor

Retort pouches offer important advantages to the processor by cost savings. The packaging materials cost for retort pouches is lower than for steel cans (comparing total package cost for a pouch and outer carton vs. a three-piece steel can, lid, and label). A roll of retort pouch stock takes up 85% less space than the equivalent number of empty cans, providing warehouse space savings on the front end of a packaging operation. The retort pouch offers savings in freight because it is lighter in weight than other packages. For example, 1000 225 g (8 oz) steel cans weigh approximately 50 kg (109 lb), compared to just over 6 kg (12 lb) for equivalent pouches. With lighter package weight, more food product can be shipped per truckload in unrefrigerated trucks. One of the principal advantages of the retort pouch is that the package is sized to the food product, not vice versa as with cans. Thus, where liquid, or brine, is not essential to the food product, much of it may be eliminated, offering even further cost and freight savings [68].

36.4.7.3.4 Advantages to the Retailer

Retort pouches can be merchandised anywhere in the store—near the checkout counter or in end aisle displays. Initially, retort pouched foods are marketed in paperboard cartons, for puncture resistance and product display; down the road, pouches may be marketed without cartons, printed with full-color illustrations, and merchandised on pegboards or special shelf units. [68].

36.4.7.3.5 Other Advantages

An environmental impact study has shown that the retort pouch, from package manufacture to consumer use, requires less energy than canned food (in cans or glass) or frozen food. The pouch also made a more passive contribution to the waste-disposal systems than other packages [68].

36.4.7.3.6 Retort Pouch Technology

A summary of the state of the retort pouch technology and its various aspects can be explained under the following headings [69]: (i) films—for a temperature range of 116 to 124°C, 9- to 25-micron polyester/9- to 25-micron foil/75-micron polyolefin (modified polyethylene or ethylene-propylene copolymers and blends) can be used. For temperatures up to 138°C, 12-micron polyester/9-micron foil/15-micron oriented nylon-6/50-micron polypropylene shall be used. (ii) Products—over 100, ranging from commodity vegetables to "ready meals." (ii) Package design—flat four seal, ranging from 10 to 100 mm × 5 mm to 175 mm × 20 mm for 150 to 300 g (5 to 10 oz) contents to 300 mm × 450 mm × 25 to 12 mm for institutional packs, for 2.5–3.5 kg (5–7 lb) contents, with folding carton or polymer bag over wrap. (iv) Pouch packaging equipment—from roll stock, intermittent motion packager for 25 to 60 pouches per minute with steam flush and closure sealing, or could incorporate in-line vacuum sealing without transfer to separate machine. From roll stock, continuous motion packager for 250 pouches per minute. From preformed pouches, filler-sealers for 25–60 per minute, can with squeezing action or steam flush for air removal. (v) Retorts—horizontal batch, water or steam-air cook, modified to assure uniform distribution of heating media; use of retort racks, with separate heating media accumulation tank; suitable for high-temperature (135°C) cooks. Continuous horizontal or vertical retorts for water or steam-air can also be used. (vi) Cartoning—standard folding carton equipment.

36.4.8 Energy Aspects of Canning

Energy analysis of the operation of the food sterilization unit is useful in two respects: first, it provides quantitative information on energy requirements of use in designing the energy generating and delivery system, and second, it evaluates the modes of energy loss. Information obtained from the energy analysis can be used for quantifying energy conservation practices [70]. Energy required for manufacturing, transporting, and processing were estimated for two alternative systems (canning line, and retort pouch line), each capable of producing about 45 metric tons of processed spinach per 8-hour shift.

The following conclusions can be drawn: (i) Container manufacturing required more than 80% of the energy required in each system. (ii) A pouch processing line will have much higher electrical requirements than a comparable canning line. However, costs associated with electrical use are small compared to total costs. (iii) The total energy requirement for a retort pouch packaging system is significantly less than that for a can packaging system. (iv) Container and energy costs for a retort pouch packaging system are significantly lower than those for a comparable can packaging system. (v) A comprehensive economic analysis must be conducted before a decision to adopt retort pouch processing technology can be made.

A dominant factor influencing total energy use in the canning industry is the heat requirements of food sterilization. The continuous cookers used in canneries are typically more energy-efficient than batch processing in retorts [71]. Energy consumption rates in operating various sterilizing equipment have been compared. The energy requirements of a rotary pressure retort, a rotary atmospheric retort, and a flame sterilizer were estimated, and the overall heating efficiency was 47.7%, 31.2%, and 27.5%, respectively [72]. The comparative costs of the heat required for sterilization of canned products by different equipment (Table 36.2) have been reported. The thermal energy balance of a stationary retort was studied [73]. Only 16.7% of the steam supplied was used in heating the cans and contents, and the remainder was lost during venting (36.4%), heating of the retort and crates (16.4%), along with the condensate in the bottom of the retort (11.2%) and through radiation (19.3%). The study indicates significant loss of steam

TABLE 36.2
Energy Costs Required for Thermal Processing of Canned Foods by Different Equipment

Processing Equipment	Comparative Costs of Heat		
Static retort	100[a]	100[b]	100[c]
Continuous rotary atmospheric retort	------	-------	64
Continuous rotary pressure retort	------	-------	46
Hydrostatic retort	20	56	------
Fluidized-bed retort	------	38	------
Microwave retort	1230	-------	------
Flame sterilization	56	-------	88

a = values in this column from ref. 103
b = values in this column from ref. 74
c = values in this column from ref. 72

during venting. Data from different canneries showed steam consumption to be quite consistent for the retorting operations, averaging 3 kg/min of steam per 24 No.2 cans. During venting, the peak of steam consumption may vary between 1135 and 2720 kg/h for a standard 3–4 crate retort, depending upon the size of the steam inlet line. The peak demand drops off to an operating demand of 45 to 68 kg/h after the vent valve is closed and the retort reaches operating temperature. A novel fluidized-bed retort [74, 75] involves heating and cooling of cans in a fluidized bed of sand or other granular material of high density. Fuel savings can be significant with a fluidized-bed retort. This is because the heating medium (usually air) does not go through a phase change and recycling of the heating medium improves the energy efficiency of the equipment.

36.5 ASEPTIC PROCESSING

36.5.1 Introduction

The development of the HTST processing methods for sterilizing in a continuous flow has brought about the need for aseptic packaging of the product. It is only through the use of aseptic packaging that the benefits of HTST treatment can be fully realized. Aseptic packaging will exhibit the greatest quality improvement over conventional canning when viscous low-acid products are processed. Many products can be commercially sterilized prior to packaging by continuous processes so that their organoleptic and nutritional quality is not significantly affected. Products such as puddings, sauces, dips, and pastes are currently aseptically processed. In the techniques applied to aseptic packaging, continuous heat exchangers can be designed so that any temperature profile may be applied. Aseptic packaging of foods is a process which enables products, sterilized in bulk or on-steam, to be filled and sealed into sterile containers under aseptic conditions. There are two reasons for its use: (i) to enable containers to be used that are unsuitable for in-package sterilization, and (ii) to take advantage of high-temperature-short-time (HTST) sterilization processes, which are thermally efficient and generally give rise to products superior in quality to those processed at lower temperatures for longer times [76].

Application of the aseptic process involves (i) sterilization of the product, (ii) sterilization of the packaging material, and (iii) maintenance of the sterility during the filling and sealing operations. The advantages of aseptic packaging of food products are that it provides a higher quality product; a wide variety of packaging materials of different sizes and shapes can be used; and there is minimum handling of the containers during the sterilization process. Also, it provides a high surface area for efficient heat transfer [77]. Aseptic processing and packaging, however, has limitations, and it does not offer advantages with all products. Some of the disadvantages that are generally cited are large capital investment, applicability to limited range of products, and requirement of relative homogeneity of the fluid and need for sophisticated instrumentation [77].

36.5.2 Sterilization Systems

The production of a sterile product by continuous flow sterilization involves (i) heating the product by passing it through a suitable heat exchanger to raise it to operating temperature, (ii) passing the product through a holding section for a predetermined time to effect sterilization, and (iii) cooling it to a temperature of 35°C or less prior to aseptic filling. The heat exchange process is limited to liquids containing small particles with a cross-section of less than about 8 millimeters. For sterilization of large pieces, special equipment is required [78, 79]. The ideal system would raise the temperature in the heat exchanger to the required value, thus eliminating the holder tube requirement. It is not possible to use a sufficiently high temperature and short residence time for this purpose with many products since (i) viscous products are difficult to heat uniformly and evenly to the operating temperature, (ii) the presence of small particles makes it desirable to impose an unheated section in order to equilibrate temperatures, (iii) the products may contain heat-resistant enzymes, which are more likely to survive processes at the top end of the temperature range, and (iv) the criticality of the process makes control difficult. The key components of the aseptic systems are the timing or metering pump, product heater, holding tube, cooler, and back-pressure valve which are characteristic of practically all aseptic processing systems. The type of aseptic processing equipment selected is basically dependent on the pH, the viscosity or consistency of the product, and on whether it contains particulate and their size.

36.5.3 Processing Equipment

36.5.3.1 Infusion Sterilization

36.5.3.1.1 Steam Injection Sterilization

This is the most rapid method of heating the product, facilitating the attainment of sterilization temperature within seconds. Combined with the rapid method of cooling by injection of the hot product into a vacuum chamber and evaporation of

an equivalent amount of water, a very high-quality product is obtained. The method is combined usually with heating and cooling in heat exchangers to the low temperature range (80°C) [80].

36.5.3.1.2 Liquid Infusion into Steam

This system involves infusion of a thin film of liquid into a steam atmosphere, facilitating rapid heating. Cooling is also achieved by infusion of the liquid into a vacuum chamber. The system is a versatile processing method designed primarily to heat and cool fluid foods within seconds. It produces the fastest heating methods, and this minimizes the flavor changes and product damage normally associated with high processing temperatures. It is especially important for low-acid products that require sterilizing up to 150°C. The system may be pre-piped and packaged or field-assembled to meet specific plant space requirements. Acquisition costs for infusion heating systems are low when high flow rates are being processed. There are few moving parts, and service costs are low. However, the method is suitable for particle-free liquids only. The heat recovery efficiency is only about 50%.

36.5.3.2 Tubular Aseptic Sterilizer

Tubular aseptic sterilizing is an indirect heating/cooling method that uses stainless-steel coiled or straight tubular heat exchangers. The tubing diameter is relatively small compared to product flow. As a result, extremely high flow velocities within the tubing maximize turbulence. High turbulence induces rapid heat transfer [20, 80]. The tubing diameter is suited to the product flow and viscosity. Tubes are fabricated into coils or bundles and placed, along with special media baffles, into stainless-steel jackets. Hot water, steam, or cold water passes through these jackets to heat or cool the product flowing within the tubes. A series of horizontal tubular heat exchangers and a vertical holder tube heat and hold the product at the required sterilizing temperature and time. For low-acid products, 150°C with a holding time of 2–4 seconds is used. High-acid product would normally be heated to around 95°C and held for approximately 30 seconds. From the holding tube the product flows to another series of vertical tubular heat exchangers for cooling. This system provides high heat-transfer rates and a scrubbing action that reduces "burn off" or fouling in the tubes, resulting in a very short processing time. This helps to preserve the natural flavor of the product. The system has considerable flexibility in the range of products it can handle and the temperature range at which a specific product is processed. They are completely self-contained requiring only product and utility hook-ups to be made during installation. There are no gaskets to replace on the high temperature side. Most systems are available with regeneration as an option. Regeneration may be as high as 85% depending on flow rates, product characteristics, and regeneration option used.

36.5.3.3 Swept Surface Sterilizer

This type of heat exchanger is similar to a tube heat exchanger but is provided with a central rotating shaft carrying a scraping device for the heated surfaces. This prevents burning and fouling of foods at the surface and also provides a mixing action. The system is used when a viscous material or one containing small, discrete particles is to be processed. Swept surface sterilizing is an indirect heating/cooling method. With the continual removal of product from the cylinder wall the product film is reduced to an absolute minimum, permitting long processing runs without product build-up on the heat exchanger wall [80]. Heat-sensitive products can be processed, and the system is versatile for aseptic processing of different products. Products can be processed over a broad temperature range and viscosity with or without particulate. The various horizontal and vertical configurations allow this form of heat exchanger to be adapted to specific systems or plant requirements. It may be used in series with other types of heat exchangers for products such as starches that might increase in viscosity due to processing.

36.5.3.4 Plate Sterilizer

A plate heat exchanger (described in the pasteurization section) can also be used for aseptic processing.

36.5.4 Packaging Systems

Aseptic packaging refers to the filling of a cold commercially sterile product under sterile conditions into a pre-sterilized container and closure under sterile conditions to form a seal that effectively excludes microorganisms. Aseptic literally means the exclusion of microorganisms from the environment. Aseptic processing is really a method of packaging because foods are not sterilized or cooked or otherwise altered by aseptic methods. Rather, they are handled or moved by aseptic methods to assure they retain the microbiological quality with which they started. In general, aseptic packaging is coupled with HTST or UHT methods of food sterilization, and the two processes are joined in a complete system to produce what are referred to in the trade as aseptically processed foods. However, the total aseptic equipment is not an actual integrated system, and the processor must purchase the sterilizer and the aseptic packager as separate units and then tie them together.

36.5.4.1 Sterilization of Packaging Materials

Package sterilization has been accomplished by using a number of methods and their combinations [81]. The most common methods are based on the use of (i) a super-heated system; (ii) hot, dry air; (iii) hydrogen peroxide; (iv) a combination of hydrogen peroxide and ultraviolet light; (v) a combination of hydrogen peroxide and heat; (vi) heat of the co-extrusion process; and (vii) irradiation by gamma rays. These methods are given in Table 36.3 [82].

36.5.4.1.1 Super-Heated Steam Systems

In this system, sterilization of the container and its closure is accomplished by the application of heat using superheated steam. The advantage of this system is that it can achieve high temperatures at atmospheric pressure; however

TABLE 36.3
Methods for Sterilizing Aseptic Packages

Method	Application	Advantages/Disadvantages
Superheated steam	Metal containers	High temperature at atmospheric pressure; microorganisms are more resistant than in saturated steam
Dry hot air	Metal or composite juice and beverages containers	High temperature at atmospheric pressure; microorganisms are more resistant than in saturated steam
Hot hydrogen peroxide	Plastic containers, laminated foil	Fast and efficient method
Hydrogen peroxide/UV	Plastic containers (preformed cartons)	UV increases effectiveness of hydrogen peroxide
Ethylene oxide	Glass and plastic containers	Cannot be used where chlorides are present or where residue would remain
Heat from co-extrusion process	Plastic containers	No chemicals used
Radiation	Heat-sensitive plastic containers	Can be used to sterilize heat-sensitive packaging materials; expensive; problems with location of radiation source

microorganisms are more resistant to super-heated steam than saturated steam.

36.5.4.1.2 Dry Hot Air Systems

Hot air sterilization has similar advantages and disadvantages as super-heated steam. There are currently no units of this type utilized in the production of low-acid foods, but the equipment has been used for the production of juice and beverages.

36.5.4.1.3 Hydrogen Peroxide Systems

A number of systems utilize hydrogen peroxide in combination with heat and/or other adjuncts. In this system, the packaging material is not metal and it comes in rolls rather than in preformed containers. The system also utilizes a different sterilizing medium. The rolls are continuously fed into a vertical machine which sterilizes, forms, fills, and seals the package. Sterilization is accomplished with a combination of hydrogen peroxide and heat. The heat necessary for sterilization may be obtained by a heated stainless-steel drum. Contact with the drum heats the peroxide and effects sterilization. Another system uses packaging material from rolls that are continuously fed into the machine which forms, fills, and seals the package. The packaging material travels through a bath of hot hydrogen peroxide which softens the material for forming. Cups are then formed, filled, and sealed with lids which also traveled through a hydrogen peroxide bath. Another system utilizes preformed cups to which a lid foil is heat-sealed after filling. The cups are fed into the machine where they are sterilized by the peroxide spray followed by heating. The lid material is sterilized by being passed through a peroxide bath. All filling and sealing are done in a chamber that is kept sterile by maintaining a positive pressure with filtered sterilized air. Another system that utilizes preformed cartons sprays the inside of the carton with low-concentration hydrogen peroxide. This sprayed carton then passes under a UV light source which acts synergistically with hydrogen peroxide in destroying microorganisms. Results of tests, using suspension of microorganisms, have shown this combination to be very effective.

36.5.4.1.4 System Utilizing Heat of Extrusion Process for Sterilization

This is a form-fill seal packaging system and relies on the temperature reached by thermoplastic resin, during the co-extrusion process used to produce multi-layer packaging material, to produce a sterile product surface. During production, the multilayer package material is fed into the machine where it is delaminated under sterile conditions. This removes a layer of material and exposes the sterile product-contact surface. The container material is then thermoformed into cups. The lid material which is also delaminated is then sealed onto the cup after filling. The sterility of the forming, filling, and sealing areas is maintained by sterile air under positive pressure.

36.5.4.2 Aseptic Filling and Packaging Machines

Aseptic filling and packaging systems can be classified into categories based on the type of packaging material and the method of forming the container (Table 36.4) [83].

36.5.4.2.1 Form/Fill/Seal Machine for Pouches

Figure 36.8 shows the principle of operation of an aseptic vertical form/fill/seal machine for three-sided sealed pouches [84]. The packaging material from reel, usually a complex multilayer material, is sterilized by hydrogen peroxide in a heated bath, which is the siphon lock to a sterile chamber with a slight overpressure of sterile air. In this chamber the film is dried, folded over a shoulder to form a tube, and sealed at the long seam. Then the tube, which is closed at the bottom by the cross seal, may be drawn to the non-sterile exterior of the chamber through a tightly fitting flexible lock. Sterile filling inside the chamber is performed using sine filler. In the tube, the contents are protected by a neutral atmosphere of sterile nitrogen, which maintains a very low oxygen concentration in the headspace of the packs. Grippers spread the sealing zones and vertically reciprocating sealing bars with cutting knives outside the sterile cabinet transport down, seal, and cut off the pouches. The pack output is 15–35 pouches/min, depending on size. Products that are, at present, filled by these machines include various tomato products, and sauces such as cheese sauce and pizza sauce with particulate. Meal constituents and curries could also be filled [85]. The filling system has CIP and SIP characteristics. Pre-sterilization of the filling system with pressurized steam and of the sterile chamber of the machine by condensed hydrogen peroxide vapors, and also heated air, is performed automatically.

TABLE 36.4
Classification of Aseptic Filling and Packaging Systems

Category	Examples of Systems
I. Metal and rigid containers sterilized by heat	
A. Steam/metal containers	Dole canning systems
	Drum fillers, e.g., scholle, FranRica
B. Hot air/composite can	Dole hot air system
II. Webfed paperboard sterilized by hydrogen peroxide	Tetra Pak (BrikPak)
	International paper
III. Preformed paperboard containers	Combibloc
	Liquipak
IV. Preformed, rigid/plastic containers	Metalbox Freshfill
	Gasti
	Crosscheck
V. Thermoform–fill–seal	Benco Asepack
	Bosch Servac
	Connoffast
	Thermoforming USA
VI. Flexible plastic containers	
A. Bag-in-box type	Scholle
	Liquibox
B. Pouches	Asepack
	Prepac
	Prodopak
	Inpaco
	Bottlepack
C. Blow molded	Serac
	ALP

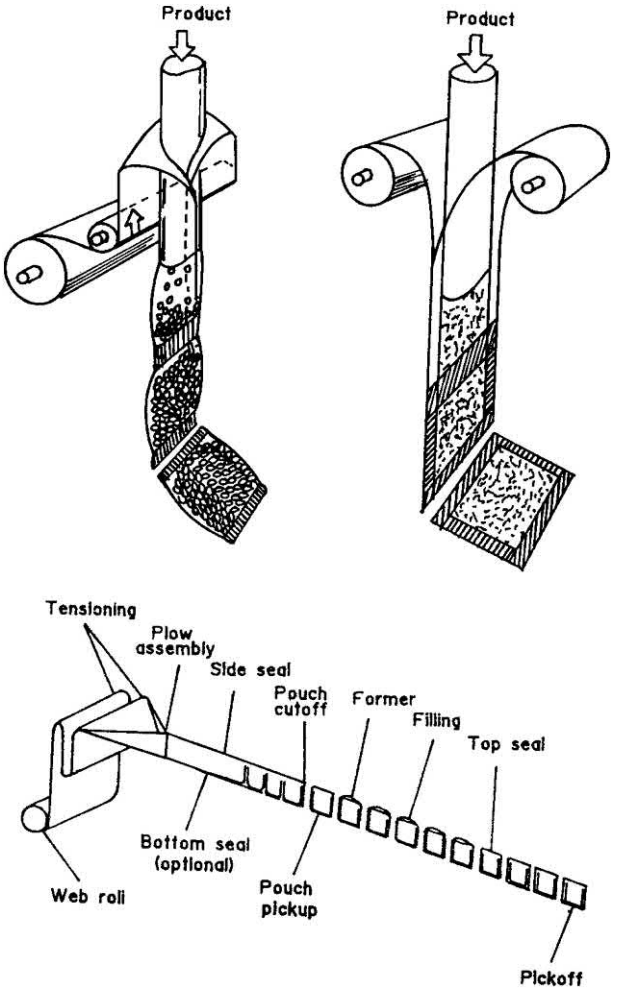

FIGURE 36.8 Form/fill/seal machines. (From Potter and Hotchkirs [84].)

36.5.4.2.2 Thermoform/Fill/Seal Machine for Cups and Trays

Film for both the cups and trays and the lid are drawn from rolls and transported into the totally closed sterile cabinet through a heated hydrogen peroxide bath. The lower film is heated locally, thermoformed with plug assistance by pressurized sterile air, and the formed packs are then filled. Filling is performed by special piston filler with reciprocating valves having cutting edges. This filler is able to deposit mixtures with particulate of a few millimeters in size. The shafts of the sliding valves and pistons penetrate the vessel for the product that has to be filled. At the mechanical drive external non-sterile air is separated from the sterile air above the product by rolling diaphragms. After filling, the lid is applied to the filled web and sealed at the rims. Headspace gas flushing may be performed. Now the webs may be transported to the non-sterile outside through a contour lock without risk of infection, where the final sealing of the packs, notching, and cutting are carried out [85].

36.5.4.2.3 Filling and Closing Line for Bottles and Jars

The containers, which are pre-cleaned and heated by a special rinser, enter the sterilizing machine in one lane, and they are sterilized in several lanes upside down, by treating inside and outside with hydrogen peroxide vapors and then drying with sterile air. The containers are inverted and transported intermittently to the piston filler. In the next stage the containers are closed with metal caps, which were sterilized with pressurized steam upon entering the machine. For liquid products, magnetic-inductive metering devices are used for filling. For plastic bottles heat-seal closures from foil are applied [85].

36.5.4.2.4 The Tetra Pak System

The principle of this system is to take the packaging material directly from the reel and to form it continuously into a tube. The tube is sealed below before filling and above after filling. The main advantage of this pack is that there is no headspace in the finished pack. The pre-creased web of packaging material unwinds over rollers, which soften the transverse crease. The web is immersed in hydrogen peroxide bath for sterilization. The packaging material is a lamination made of pare, foil, and polyethylene. This combination gives a light and gas barrier, strength, and heat-sealing properties [20, 76]

36.5.4.2.5 Conoffast System—Continental Can Co.

The system employs prefabricated and sealed pouches that may be internally sterilized by any one of four methods.

Empty pouches are fed through an ultraviolet chamber to minimize external pouch contamination and then filled using hypodermic needles in a superheated steam atmosphere. The needle is withdrawn, and the puncture area is heat sealed. Ionizing radiation has been suggested to sterilize the pouch material surfaces. Steam pressure sterilization restricts the packaging materials to those which can resist the temperatures. To sterilize the filling and sealing area and to sterilize the needle, superheated steam at 145°C can be used [20, 76]. The Conoffast system also utilizes the basic package forming–filling–sealing system. Sterilization of the inside of the package in this system is based on the high temperatures generated within the thermoplastic resins during the extrusion process used to produce the multi-laminated packaging material. During the aseptic packaging operation, the top plastic sheet is delaminated, exposing a sterile surface of the packaging material to the food in a sterile environment within the Conoffast unit. Before filling, the packaging material is thermoformed into cup-shaped containers. Sterile food product is filled into the package under sterile conditions. Then the packages are sealed with packaging material from which one layer has been delaminated, again exposing a sterile surface to contact with the food. Sterility during the forming, filling, and sealing operations is maintained by performing these operations in a sterile environment under positive pressure.

36.5.4.2.6 The Combibloc System

In this system premade carton blanks are used, which are die-cut, creased, side-seamed, and printed at the factory origin. This facilitates a more perfect flame-welded seam, thus ensuring good integrity of the seal, and the packaging machinery can handle different sizes with a simple height adjustment. A carton blank is drawn from the magazine by suction pads and placed on a mandrel. The sealing surface at the bottom of the carton is softened by hot air. The bottom is folded, pressed, and sealed against the end face of the mandrel. The top is pre-folded and then passed on to the aseptic zone where it is sterilized by hydrogen peroxide spray. After filling, the package top is folded and sealed by ultrasonic welding [76].

36.5.4.2.7 The International Paper System

In this system the packaging material is taken from a reel. From the reel the web passes through a series of scoring rollers into a hydrogen peroxide bath for sterilization. The horizontal seals are made by alternating jaws and an induction heater. Individual packages move to the final folding and sealing station for sealing of top and bottom flaps [76].

36.5.4.2.8 The Gasti System

This system operates with preformed cups made of plastic or aluminum. This facilitates greater flexibility in the operation and allows container quality to be approved in advance. In operation, preformed cups are dispensed from a magazine and are sterilized using hydrogen peroxide vapor. The cups are then filled in the sterile section and are sealed with presterilized lids [76].

36.5.4.2.9 The Liqui-Pak System

This system uses a combination of two sterilizing methods to obtain aseptic packages viz. hydrogen peroxide and ultraviolet light. This approach has a synergistic effect which results in a more effective bactericidal action than high concentrations of hydrogen peroxide and ultraviolet light used individually. The cartons travel through the sterile area against a flow of filtered air. The filler is unique with a plastic bellows mechanism for sterile dispensing of the product. After filling, the gable-carton is heat-sealed in a conventional manner [76].

36.5.4.2.10 The Metal Box "FreshFill" System

This system uses preformed cups sterilized by hydrogen peroxide. The product is filled by a multi-head filler, with the filling chamber isolated from other machines areas by sterile air overpressure generated by an ultrafilter. The filled cups are sealed using a pre-sterilized foil material and stamped out in the conventional manner. Before start-up, the filling chamber, fillers, and supply line are sterilized with steam at 130°C for 20 minutes [76].

36.5.4.2.11 Avoset System

This system packs fluid products in glass and cans and then in aerosol containers. The system sterilizes containers and product separately and brings them together in a sterile environment. The entire piece of equipment is placed in a controlled environment, and all critical elements are sterilized. An operator is present to monitor the equipment. Although sterility is assured, distribution under refrigeration to retard biochemical changes is generally recommended [20, 76, 86].

36.5.4.2.12 Manton-Gaulin (Pet Inc.)

The system is basically a glove box. Polyethylene bags are sterilized in the glove box with an ethylene oxide mixture and are heated to 49°C for 6 hours. After sterilization, the gas is replaced by sterile air. Sterile mix is aseptically pumped to the filing nozzle within the glove box. Using glove box techniques, the operator fills one bag at a time through the rigid spout. The spout is heat sealed with a laminated foil material. The filled bag leaves the glove box through a chlorinated water trap and is dried outside of the aseptic filling area [20, 76]. A Scholle container with a semi-automatic aseptic system for filling 6-gallon polyethylene bags has been used. Sterile conditions are maintained by pressurized sterilized air and a continuous spray-mist of chlorine solution over a hinged-cap fitment on the bag during filling.

36.5.5 Quality of Aseptically Processed Foods

The basic consideration for sterilizing at high temperatures for a short time is that for each 10°C (18°F) increment in temperature, the sterilization required for destruction of bacteria is reduced by a factor of ten, while the rate of destruction of nutrients and of other chemical reactions affecting product color and flavor increases by a factor of approximately three. This is called the "z" value. /The higher the sterilization temperature, the larger the difference between rates of

destruction of reactions. This is the concept upon which the most important advantages of aseptic processing and packaging systems are based. Because of this the organoleptic and nutritive characteristics of the products processed by aseptic processing systems are retained as compared to those processed by other systems, such as conventional canning [87]. Chemical and flavor changes during high-temperature heating are particularly severe in low-acid foods, which require more severe heat treatment to be sterile. At higher processing temperatures, bacterial spore destruction is much faster than the destruction of food constituents. Foods processed by aseptic processing are better in color and higher in thiamin than those made by conventional canning processes. Aseptic processing is used commercially in the dairy industry and to fill fruit juices and purees, pea soups, sauces, tomato paste, etc.

The effect of HTST sterilization on the nutritive value of meat products has been a subject of great interest. Protein quality may be degraded by the destruction of one or more of the essential amino acids, formation of inter- and intramolecular bonds resistant to digestive enzymes, and alteration in the rate at which the various amino acids are released from protein. This results in a mixture of amino acids that may be less efficient for metabolism and assimilation. The essential amino acids tryptophan, methionine, and lysine are also destroyed by HTST sterilization. Destruction of methionine and accumulation of sulfur dioxide are the main chemical indices of the effect of cooking meat proteins at 120–150°C. Protein denaturation is not the only change that occurs during heat processing. Hydrolysis of proteins and polypeptides also takes place. Collagen, elastin, and reticulin compose the connective tissue of meat and are insoluble in water and salt solutions. Collagen is transformed by heating into soluble gelatin. In this conversion, some of the crosslinks are broken, resulting in shortening and disorganization of the protein chains. The conversion also accounts for higher protein solubility in the processed meat products. Aseptic strained meat has been shown to be more nutritious and higher in thiamin retention. Aseptically processed meat and vegetable products lose thiamin and pyridoxine, but other vitamins are largely unaffected. There are negligible vitamin losses in aseptically processed milk, and lipids, carbohydrates, and minerals are virtually unaffected. Riboflavin, thiamin, pantothenic acid, biotin, nicotinic acid, and vitamins B_6 and B_{12} are unaffected. Nutrient losses also occur during periods of prolonged storage, and these should also be considered when assessing the importance of sterilized foods in the diet.

36.5.6 Nutritional Aspects of Aseptically Processed Foods

The use of HTST processes is particularly adaptable to aseptic processing. The destruction of nutrients during the thermal processing is dependent on (i) time–temperature treatment used as the basis of the process and (ii) the rate of heat transfer into the product. In an aseptic processing system, as the processing temperature is about 150°C for a very short period, the nutrient retention is greatly enhanced. The effects of heat treatments of equal microbial lethality on selected food constituents including nutrients, color, proteins, and flavor compounds have been reported [88]. The retention of vitamin C in tomato juice improves during HTST processing. For natural products containing enzymes the limitation of the benefits of HTST processing occurs when the basis of the process shifts from microbes to enzymes at a temperature of 130 to 145°C for shorter periods.

In an evaluation of HTST aseptic processing [89] it was found that thiamin retention was significantly greater in HTST products than conventionally canned and retorted products. For pyridoxine, the benefit of HTST was not as evident, as destruction of pyridoxine is not as temperature-dependent as that of thiamin. HTST aseptic processing also results in significant improvements in organoleptic qualities [90]. Most of the reports on the effect of thermal processing on nutrients only contain information on the content of a specific nutrient after the thermal process and give the percentage retention or loss of the nutrient. As there are numerous processing methods and time–temperature possibilities for accomplishing commercial sterilization, it is improper to assume that the nutrient losses reported in the literature represent the average or norm for the industry. Hence, the data on such nutrient losses are of limited value but can be used as a guideline for selecting an optimum process schedule [90]. Nutrient losses range from 0 to 90%, depending on the nutrient and the product. These losses represent the sum of the losses during the entire processing including blanching. In some of the studies [91–93], the temperature for sterilization of foods has been optimized to maximize retention of nutrients or minimize the production of an undesirable product.

36.5.7 Packaging of Aseptically Processed Foods

36.5.7.1 Introduction

Packaging of aseptically processed food is the most critical key for a successful operation. The product has to be packaged in the form desired, which will yield the benefits anticipated after the product has been sterilized. This includes process design, process equipment, formulation, and raw material quality. Besides the machinery used, the materials used for the container and the closure and the sterilants that can be used with these materials are important. The aseptic packaging system must be capable of filling the product produced by the UHT or HTST system in an aseptic manner and sealing the container hermetically so that sterility is maintained throughout the handling and distribution process. Thus, the system must be capable of (i) being connected to the processing system in a manner that enables aseptic transfer of product to take place, (ii) being effectively sterilized before use, (iii) carrying out the filling, sealing, and critical transfer operations in a "sterile" environment, and (iv) being cleaned properly after use. The packages in use vary from traditional tinplate cans and glass bottles to the non-rigid and semi-rigid containers based on thermoplastics or combinations of thermoplastics with paperboard and metal. The type of container used will be influenced by the nature of the contents, cost,

and its acceptability to the consumer. The relatively low cost and wide acceptance by the consumer are the main reasons for the recent proliferation in the use of the carton-type packs for fruit juice, juice-based beverages, and dairy products. In addition to systems producing packages intended for sale directly to the public, bulk packaging installations are used for conserving raw materials or intermediates intended for reprocessing or for use in catering establishments.

Many systems have been proposed for the aseptic packaging of food, but some authorities consider that not all those proposed meet the criteria for full asepsis. Thus, they should be more accurately described as ultra-clean fill machines and used as a means of extending the shelf life of products distributed through the chill chain. Some systems, by their nature, restrict the packaging material to simple monolayer plastics, and many foods packaged in these materials have a limited shelf life due to their poor oxygen barrier characteristics. Others are able to employ multilayered barrier plastics or to include aluminum foil as a component, and in such cases, shelf life is much improved. Containers made from glass or metal of adequate thickness may be considered impermeable [76]. The method of sterilizing the package, or material from which it is formed, is important in retaining the characteristics of the packaging material. Sterilizing processes may alter the characteristics of the package, or material, and render it undesirable for packaging the food product. Obviously, the material must perform and produce the results desired. Some of the factors that should be considered include (i) performance with the food (gas transmission, water transmission, absorbance—packaged product flavors, odors, colors, and vitamins being absorbed by the container material, adsorbance—a few molecules being extracted from the package material and being held by the product, combinations of absorbency and adsorbancy, chemical inertness to the food, or desirable reactions with the food, sterilization, wet ability, temperature limitations, and inertness to the sterilizing agents, can could be laminated or special surfaces applied, material handling characteristics (empty)), (ii) cost, (iii) form and mechanical characteristics (can it be molded, size limitations, shape limitations, easy-to-open closures available, temper-proof closures or temper-evident systems available, configuration after storage, material handling characteristics and suitability for use with conveyors, labelers, casers, and over cappers), (iv) shipping and handling (toughness or strength, type of over wrap or cases required, fillers between packages in the cases required), and (v) compliance with regulations (Food safety FDA, FSIS, 3A, PMO etc.,) [94].

The materials that are used for aseptic packages are practically infinite. This is because the use of laminated materials, using plastics as well as metals, and the development of new plastic materials and alloys, occurs regularly. There are also variations within a defined product so it may vary from different manufactures, even though generally it is the same. For example, paper from a supplier may vary from paper that comes from another vendor. The same is true for metals such as steels and aluminum, plastics, and laminates, etc.; these variances can cause the product to change over a short or long period of time. Some of the common materials used for aseptic packages include the following: (i) stainless steel (bins, tanks, and rail cars), (ii) carbon steel (cans and closures), (iii) aluminum (cans and closures), and (iv) plastics (cups and square/rectangular packages) (acetal, nylons (66 or others), polypropylene, polyester, polycarbonate, acrylic, acrylonitrile-butadiene-styrene (ABS), polyvinyl chloride (PVC), polystyrene, high-density polyethylene, low-density polypropylene, ethyl vinyl acetate (EVAL), ethyl vinyl alcohol (EVOH), polyvinylidene chloride (PVDC), paper, paper-based laminates, plastic-based laminates) [94].

It should be taken into consideration that new materials are constantly being proposed for approval by the FDA for aseptic packages with specified types of sterilants. Also, certain laminates that exist today are made in a single polymer form; hence, a resin may contain three or four different polymers that have combined properties. The reason for manufacturing laminates out of various materials is to build in to the packaging material(s) those properties that are most critical for the food product over a period of time (which may be short, medium, or long), and it will be either inert to the product or react with it in a favorable way, such as tin cans reacting with citrus juices. The problem of the food product compatibility with container materials over a period of time should be briefly addressed. This is an area that has not received the attention it should have from packaging manufacturers or food processors.

Materials can absorb desirable components from the food, thereby causing the food to have less flavor, color, odor, and nutrients. Another condition that exists is that the packaging materials can actually have certain molecules stripped from it, thereby changing food product's color, flavor, odor, and vitamin content. Also, there are combinations of the two where the material used for packaging reacts with the food to change its properties, and in turn the food reacts with the packaging material and loses some of its desirable characteristics. This situation is never ending and may actually accelerate as color changes occur [94].

36.5.7.2 Aseptic Packaging

In general, aseptic packaging is coupled with HTST/UHT methods of food sterilization, and the two processes are joined in a complete integrated system to produce aseptically processed foods.

36.5.7.2.1 Drums

The drum is placed under the filling chamber and then raised up to seal against a gasket. Saturated steam is introduced into the drum, and the interior of the drum is pressurized with steam. After a total 2.5-minute cycle, a filling tube lowers into the drum and dispenses sterile product to fill the drum. The filling tube retracts, and a sealing head with magnetically attached lid swings over the drum. Steam pressure is employed to apply pressure to crimping jaws to fix the gasketed lid into place. Alternatively, an empty drum and cover are placed in a filling retort, and both are steam sterilized under pressure. Sterile product is then filled into the drum,

and the lid is placed. The drum is removed, and another cycle is initiated. The capacity of the system with two filling retorts is 24 drums per hour [20, 95]. The advantages claimed of the drum system are lowered shipping weight because one large reusable container is used rather than 75 No.10 cans plus 12 to 13 corrugated cases, product recovery is higher because there is far less surface area to drain, And further, the labor cost of emptying one 200 L container is significantly lower than the cost of opening 75 cans.

36.5.7.2.2 Tanks

Instead of pulping, finishing, and concentrating into paste for bulk handling, tomatoes are simply chopped, sterilized, and filled aseptically into 380 L (100-gallon) tanks. Tomatoes are washed, chopped into chunks, heated in tubular heaters, cooled, deaerated, and subsequently filled into tanks under a nitrogen blanket. Storage tanks are galvanized steel, lined with a baked-on epoxy coating. Tanks are chemically sterilized before filling [20]. The basic objective is to provide tomato processors with a large source of raw material that can be converted into many different products over a long period. Among the products that can be made from chopped tomatoes are paste, puree, sauce, catsup, pizza sauce, juice, and chili sauce.

36.5.7.2.3 Glass Containers

Aseptic packaging in glass containers has not been broadly successful on a commercial basis. Juice is heated to 93°C and held for 9 seconds and cooled to 20°C in a heat exchanger. Bottles of 1 and 2 L are cleaned by rinsing with water. After washing, bottles are discharged into a closed area blanketed with 99.9% sterile, dehumidified air. The filler is sterilized with boiling water prior to operation. Closures are steam sterilized and taken into the cleanroom. Sterile juice is filled into the "sterilized jars" and capped in the clean area. The product, known as aseptic cold-pack juice, is reported to have a long shelf life under 10°C [20]. At room temperature storage, hot-filled (conventional) juice has an acceptable shelf life of one month as compared to three months for cold-filled juice. The basic problem with glass is that the maximum temperature differential glass containers can withstand is approximately 15°C. Thermal shock between inner and outer surfaces leads to unequal thermal expansion sufficient to crack the glass.

36.5.7.2.4 Plastic Containers

The concept of aseptic packaging holds that pre-sterilized product is filled under aseptic conditions into a pre-sterilized container. As long as the container can exclude microorganisms and prevent the passage of gas, the container material need not be rigid. Metal in heavy gages and glass are both fabricated into rigid containers. There are no rigid plastics that are employed commercially for sterile packaging. All plastic materials are partially permeable to moisture and gas. Because of implied low strength characteristics, food products that have been sterilized are packaged in semi-rigid materials. Paperboard with appropriate coatings is also utilized to form semi-rigid packages [20]. Two basic types of semi-rigid systems are in commercial use: (i) thermoform, fill, and seal, and (ii) preformed cups, fill, seal [20].

36.5.7.2.5 Flexible Packages

The Tetra Pak AB system is often considered as a flexible packaging system. Technically, it is not a flexible packaging system because it is made of paperboard rather than a true flexible material. The polyethylene pouching material is sterilized using a hydrogen peroxide bath. The vertical form, fill, and seal milk pouching equipment is converted for aseptic packaging [96].

36.5.7.2.6 Reclosable Aseptic Packaging System

Reclosing is useful for products such as milk, fruit juice, and wine. Shaking is important for products such as pulpy fruit juices. The system consists of a closure with two parts. The visible part is a rectangular polypropene chassis with a cap that snaps open and shut on a hinge. The device is glued to the top corner surface of the pack with hot-melt adhesive [97]. Underneath the cap is the second part of the system—an aluminum pull tab that covers the pouring hole and provides good tamper evidence. The pull tab, which is welded to the inner liner of the package, is easy to peel off. It reveals a long, pear-shaped hole that has excellent pouring characteristics. The reclosing system is extremely sanitary, unlike "punch" style cap devices that require users to poke a finger into the product in order to open the package. High-quality long-life product is achieved by the aseptic filling process. This package provides easy stackability, space saving in terms of transportation (both filled and unfilled cartons), low weight, cost savings in carton material production, and a high level of environmental friendliness. The reclosable system increases the intrinsic value of the carton and provides the consumer with easy opening, spill-free pouring, and reliable reclosure as well as hygienic handling. After reclosure, the product is protected and can be shaken as necessary. The product quality remains unimpaired, protected from foreign flavors. The carton with the system fitted is ideal for refrigerator storage. The new carton image is completed by the slim, elegant shape suggestive of high quality.

36.5.8 Energy Aspects of Aseptic Processing

Energy aspects of aseptic processing have been widely carried out on milk, and most of the data available are on milk products. The energy requirements of aseptic processing units containing steam injection without regeneration, and tube heat exchangers as applied to the processing of milk have been evaluated. For steam infusion the system consumed about 1000 kJ/kg of milk [98]. For tube heat exchanger the system consumed about 400 kJ/kg milk [99]. The total energy requirement in the HTST system for milk processing was about 225 kJ/kg of milk. The break-down of the energy was 98 kJ/kg milk thermal, 30 kJ/kg electrical, and 97 kJ/kg refrigeration. The steam-infusion system requires about 360 kJ/kg of milk as it is a direct contact heating system and is requires more compared to indirect heating as in a tube

heat exchanger [100]. Cooling below 30°C is not required for aseptic processed milk. This saves more energy and requires about 100 kJ/kg of milk. In addition, the aseptic processing of products does not require post-processing refrigeration resulting in further savings of 900 kJ/kg of product [100]. The most widely used heat exchangers in aseptic processing of foods are plate and tubular types. If direct heating and cooling of product to sterilizing temperatures are used, the energy consumed is considerable. Therefore, the use of regeneration of heat is important [101]. The plate heat exchanger holds a distinct edge in this regard; regeneration efficiencies of 90% (direct) or 85% (indirect) have been used. Tubular units can achieve regeneration by indirect methods, whereby a secondary water flow exchanges heat from the preheater tubes to the cooler tubes. Indirect regeneration requires four times the surface as does direct, for the same heat recovery; as a result, regeneration efficiencies over 70% are rare. Steam injection/infusion systems are not efficient: for example, with milk duties regeneration accounts for only slightly more than 50% of the total heat input. Regeneration with swept surface units, while not impossible, is not used due to capital cost consideration.

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37 Cooking and Frying of Foods

M. N. Ramesh and Mohammed Al-Khusaibi

CONTENTS

37.1 COOKING

Some foods are consumed raw, and others are cooked. There are several reasons for this. Cooking improves the flavor of the food, for instance, the flavor of uncooked flour or sour apples is not very pleasant, but when the flour has been converted into bread and the apples stewed with sugar, their flavor is much improved. On the other hand, fresh strawberries are not cooked as this would spoil the delicious flavor of the raw fruit [1]. Cooking may also improve the attractiveness of food. Eating a raw chop is not much relished, but after cooking, it has an appetizing appearance and a good smell. Even more important, cooking may make food more digestible. It would be difficult to eat the flesh of a raw chop (or uncooked flour) even if you wanted to, but after cooking, it is much more tender and so easier to chew and digest. Finally, cooking may improve the keeping quality of food and make it safe. For example, milk may be boiled to delay the souring process and kill bacteria. The preservation of food by heat treatment is quite distinct from cooking.

Cooking is only one part of food preparation. Apart from the actual cooking process, ingredients may have to be blended together, and they may need special preparation by soaking, sieving, or chopping. Seasoning, spices, herbs, and sauces may be used to improve the flavor and color, and garnishes may be added to improve attractiveness, and texture may be improved by grinding, mashing, or mixing.

37.2 COOKING METHODS

Cooked food is food that has been changed in various ways by heat treatment. The heat may be applied in a number of ways; it may be dry or moist, it may be applied by means of fat or by infrared radiation [1] (Table 37.1).

TABLE 37.1
Summary of Cooking Methods

Method of Heating	Method of Cooking	Description
Dry heat	Baking	Cooking carried out in an oven
	Roasting	Baking with the addition of fat
	Grilling	Using direct radiant heat
Moist heat	Boiling	Using boiling water
	Stewing and poaching	Using hot water below its boiling point
	Steaming	Using steam from boiling water
	Pressure cooking	Using water boiling above its normal boiling point
Fat	Frying	Using hot fat
Infrared	Similar to rapid grilling	Using infrared radiation
Microwave	Similar to rapid grilling	Using microwaves

37.2.1 Dry-Heat Methods

37.2.1.1 Baking

When food is cooked in an oven, it is said to be baked. Baking is a rather slow method of cooking, but it has the advantage that large quantities of food can be cooked evenly. Baking is similar to roasting as both use dry heat. It is common to say that baking denotes the dry-heat cooking of a flour mix (starch-based formula) while roasting is the dry-heat cooking of protein-based food products. Baking depends on a list of defined ingredients and a set of instructions about combining these, which are called the formula [2]. Every ingredient in the formula has a specific role (e.g., structure builder, tenderizer, flavor enhancers), and the accuracy of these ingredients is crucial to achieving the desired quality. Some baked products are leavened by either yeast fermentation (yeast) or chemical gas production (baking soda or baking powder). In addition, the proper oven temperature is significant so every ingredient functions properly at the desired timing during the baking process. Baking temperature highly depends on the product being baked which depends on the ingredients and their proportions in the formula.

37.2.1.2 Broiling and Grilling

Broiling and grilling are other methods of applying dry heat. In broiling, the food is placed beneath a red-hot source of heat, usually a glowing metal grid. Radiant heat is directed onto the surface of the food which is rapidly heated. Broiling heat is applied to the top surface of the food, and the food should be turned from time to time. Infrared grilling makes use of heat rays which have longer wavelengths than visible light. Some of the radiation used in normal grilling is of this kind, but in infrared cookery, the proportion of infrared radiation is much increased, and this reduces cooking time to such an extent that a steak, for example, may be cooked in a minute. Grilling is similar to broiling but the heat source comes from beneath the food. The heat sources can be coal/flame or radiant heat.

37.2.2 Moist-Heat Methods

Although cooking with water involves using low temperatures, it is a relatively quick method of cooking because water has a great capacity for holding heat and for transferring this heat rapidly to food by means of convection. In moist-heat cooking, food is heated by either water or steam. Boiling uses boiling water; simmering uses water near, but below the boiling point and is similar to both stewing (for meat and juice) and poaching (for fish). Boil-in-the-bag cooking uses boiling water indirectly, but because the food is sealed in the bag this method prevents loss of flavor and soluble nutrients into the cooking water. In steaming, steam is used directly to heat the food or indirectly to heat the container. Although steaming is slower than boiling, cooking may be sped up by the use of a pressure cooker, in which steam is produced at higher than normal pressure. The increase in pressure raises the temperature at which water boils, so the cooking temperature is increased and the cooking time is reduced.

In essence, a pressure cooker is a pot with a well-fitting lid arranged so that steam can be safely generated under pressure. The pot and lid lock together by means of a groove to make the cooker pressure-tight. The food to be cooked and the required amount of water are put into the pot, which is then closed. When the closed pot is heated, air is driven out through the air vent until the cooker is full of steam. In pressure cookers with a pressure indicator, the vent then closes and pressure builds up to the value required. Slow heating only is then needed to maintain this pressure, which is shown by the pressure indicator. Should the pressure rise too much, steam automatically escapes through the air vent. The fusible plug is a second safety device; this will melt if the cooker overheats or boils dry.

37.2.3 Frying

Frying involves the immersion of food pieces in hot oil. Oil has a lower heat capacity and hence heats up faster compared to water. Different oils can be used for the different types of frying; the choice depends on the smoke point of the oils. Oils with high smoke points such as sunflower, corn, soybean, palm oil, and palm olein are preferred for deep frying. Frying is popular due to the development of unique organoleptic characteristics in food being fried. The process is characterized by mass and heat transfer.

37.2.3.1 Deep Frying

In this method, food is fully immersed in oil, which is heated to frying temperature (160 to 220°C). This results in a pleasant flavor, golden brown color, crispy outer texture, and cooked core. Different oils can be used for deep frying depending on their smoke point. The process is characterized by mass and heat transfer. A substantial amount of oil is transferred from the frying bath to the food, and water is also transferred from the food to the water bath. This results in a food with a new moisture and oil content compared to the raw material.

37.2.3.2 Shallow Frying (Pan Frying)

Food is partially submerged in a shallow pan with enough oil to cover the bottom of the pan. Although such a method is quick, the heating of the food is uneven and it should be turned from time to time. Lard, drippings, and vegetable oils (e.g., olive oil, corn oil, and cottonseed oil, often blended together) are best for shallow frying.

37.2.4 Microwave Cooking

In ordinary cooking, heat is applied to the outside of food and it gradually penetrates into the inside. In microwave cooking, the heat is generated within the food. In a microwave oven, microwaves penetrate the food and are converted into heat within the food. Thus the whole food heats up very quickly. Microwaves can only penetrate food to a depth of 3–5 cm; thus, small pieces of food are cooked very quickly. Larger pieces of food are cooked more slowly, however, because where the microwaves cannot penetrate the food is heated by conduction.

The great advantage of microwave cooking is its speed. For example, a fish fillet is cooked in only 30 seconds, a chop in 1 minute, a portion of chicken in 2 minutes, and a baked potato in 4 minutes. For this reason, microwave ovens can be especially useful in places such as canteens, snack bars, and hospitals, where food often has to be kept hot for long periods before it is eaten. Microwave ovens also have some disadvantages. For instance, cooking times must be carefully controlled. In addition, food does not turn brown or develop crispness in a microwave. Crispy bacon or conventional-looking brown crusty chops need to be finished off under the broiler.

37.2.5 Slow Cooking

The use of microwave ovens and pressure cookers is intended to speed up cooking, but recently cookers designed to slow down the cooking process have been introduced. Slow cookers are electrically heated and made of a material with good insulating properties such as earthenware so that heat transfer to the food is slow and a steady temperature is maintained during cooking. Slow cookers work on low power (1 kW) so that the cooking temperature remains below 100°C. The result is that food is cooked at a low, even temperature over a long period, usually 4–6 hours. Slow cookers are much more economical to operate than conventional ovens. Slow cooking is ideal for cooking casseroles, stews, and cheaper, tougher cuts of meat so that they become tender, facilitating reduction in weight loss by evaporation. It also prevents loss of juices because the slow cooker is sealed and no moisture escapes.

37.3 APPLICATION OF HEAT

The three common systems of heat application are (i) indirect heating by combustion gases conducted through flues, radiators, or past surfaces of the baking chamber such as the underside and backside of the chamber, (ii) semi-direct heating in which part of the combustion gases are forced into baking chamber to create pronounced convection currents, and (iii) direct heating using electricity or gas with ribbon-type burners [3].

A fourth, indirect method that utilizes high-pressure steam tubes is used to a very limited extent. The indirect firing system utilizes isolated combustion chambers from which the hot combustion gases are circulated by either suction or pressure through a bank of radiator tubes and are either returned to the combustion chamber or vented to an exhaust flue. The radiator tubes, which transverse the baking chamber, give up their heat by radiation and convection to the baking chamber. Depending upon the size of the oven, several combustion chambers or unit heaters may be used, each equipped with a burner, circulating fans, ducts, radiator tubes, and temperature controllers. The radiator tubes can be so arranged that radiated heat is applied to both the top and bottom of the pans. Because of the inherent limitation of the radiating heating surface that can be designed into an oven, the heating efficiency of indirect-fired ovens is generally less than that of direct-fired ovens.

The semi-direct firing system resembles the indirect system in using a separate combustion chamber and relying on radiator tubes to carry the hot combustion gases to the baking chamber. Here, however, the radiators are provided with either thin slots or small holes so that the hot gases are forced into the oven chamber, thereby creating extensive convection chambers, which are further augmented by a forced draft. In this system, the baking effect is produced by convectional and radiated heat, and in this way, advantage is taken of the special benefits offered by both methods of heat transfer.

In direct gas-fired ovens, ribbon burners are placed directly in the baking chamber, crosswise to the travel of the oven trays or conveyors. Originally, an air–gas mixture was supplied to the burners by an aspirator in which the combustion air was drawn into the mixing unit by the vacuum created when the gas was forced through the gas orifice under pressure. Each burner has its own gas control valve which must be manipulated individually for heat control. The aspirator system has a number of disadvantages, principal among which is its tendency to flame-out under certain operating conditions, thereby creating hazardous oven conditions.

A safer and more efficient firing method has been devised in which gas and air are mixed prior to being fed through a common header to the individual ribbon burners. In this system, filtered air is supplied to an air–gas mixing unit. One such unit is provided for each oven zone consisting of as many as 15 or more ribbon burners. In this way, all burners in one zone can be throttled uniformly by means of a single zone control valve. The unit mixer in which the air and gas are mixed is under pressure for each individual ribbon burner. With this system, the gas and air headers for each top and bottom heat zone are provided, respectively, with a zero gas regulator and an air valve controlled by an automatic temperature controller. The gas regulator and air valve operate together to uniformly supply the correct amounts of gas and air to the unit mixer in the zone. For balancing the heat distribution across the width of the oven, flame distributor-type burners are used, which provide a three-zone control of the burner flame across the oven.

The direct gas-fired system is the simplest and most efficient method of oven heating. By proper design and distribution of ribbon burners, uniform gas consumption can be obtained throughout the oven. The velocity of the combustion gases coming out of the burner ports, together with the natural convection currents created by the generated heat, supply sufficient turbulence to produce fairly uniform temperatures throughout the chamber as long as the size, number, and location of the burners are adequate and the control equipment is correctly engineered and installed. In some direct-fired ovens, forced convection is applied to obtain a more efficient utilization of the heat energy by recirculating the chamber atmosphere past the ribbon burners rather than venting it to outside.

In the steam-tube system, oven heating is performed by a series of high-strength, hermetically sealed tubes that are partially filled with water or a heat-stable liquid possessing a high boiling point. The tubes are installed in the oven so that a short portion of each tube protrudes into the combustion chamber, where direct heat is applied to cause the water to vaporize into high-pressure steam. The balance of the tube extends into the baking chamber. The tubes are slightly inclined toward the fire box so that steam condensate returns to the heated ends to be revaporized. Because saturated steam at atmospheric pressure has a temperature of 100°C, and because baking temperatures are normally within the range of 182–232°C, it is evident that considerable pressure must be developed in the steam tubes to attain baking temperatures.

Heat transfer in this system is principally by radiation and is, hence, governed by the physical law that states that the heat rays radiated from any one source vary inversely to the square of their distance. Therefore, the steam tubes must attain much higher temperatures than the actual baking temperature. The magnitude of the pressure developed within the tubes is indicated by the fact that a temperature of 335°C requires a steam pressure of 13,800 kPa. The use of steam tubes as a method of oven heating has been largely discontinued as it is relatively inefficient and lacks the necessary flexibility of heat control.

37.4 COOKING EQUIPMENT

The objectives of the cooking process are to reduce the moisture content of the product mixture, to melt, to solubilize, to caramelize, if necessary, and to invert. There are many types of cooking equipment, which may be classified in several ways for the convenience of discussion. According to the working mode, they are batch cookers, semi-continuous cookers, and continuous cookers. According to the method of heating, they come as direct-fired cookers or steam-jacketed cookers. According to the working pressure, they are atmospheric cookers, pressure cookers (steam-injection cookers), and vacuum cookers.

37.4.1 Batch Cookers

37.4.1.1 Steam Kettles

Food preparation of liquids and pumpable foods (including particles) is carried out in kettles or in heat exchangers. Kettles are used in batch operations. Heat is supplied to the foods through the kettle wall from condensing steam in the steam jacket covering most of the kettle wall. The jacket is equipped with an air-venting valve and a condensate-removal system with a steam trap. The food is transported to and from the wall into the bulk of the food by a flow created by the density differences between hot and cold products or by mechanical agitation. The free convection flow develops easily when the heat supply is sufficient for a rapid steam pressure build-up in the jacket. In the flow pattern, only a thin layer of the fluid close to the wall will be involved in the heat transfer. The heated fluid will rise to the top of the volume and spread over the top surface. The major part of the volume will slowly fall towards the bottom, where it will contact the heat-transfer surface again.

Models of steam kettles differ in relation to (i) depth, which may be deep or shallow, (ii) steam jacketing, which may be full or two-thirds, (iii) mounting, which may be on legs, a pedestal, or wall-mounted, (iv) type, such as tilting or stationary, and (v) source of steam, which may be direct or self-generated. The materials commonly used are aluminum and stainless steel; the finish may be dull or polished. All steam equipment should have safety valves and pressure gauges. Those having self-generated steam should have an automatic low-water cutout and a thermostatically controlled cutout heat. Tilting kettles should have a secure device for stopping them at any desired degree of tilt.

37.4.1.2 Steam Cookers

Steam cookers vary in (i) number of compartments (single or in stacks of two or three) and size, (ii) source of steam, (iii) type of base, and (iv) design. Steamers should have heavy-duty gaskets. Cooking containers must be suitable for the material to be cooked, should minimize handling, and should permit suitable load size for workers to lift. The use of serving pans may often minimize transfer.

37.4.1.3 Vacuum Cookers

This system is used for the production of hard candy, jelly, gum candies, and low-boiled sweets. The main components of the vacuum cooker consist of a cooking chamber, a kettle under it with a vacuum facility, a stirrer inside the cooking chamber with a variable-speed drive, a vacuum pump with a spray condenser and control system. The finished product is discharged into the kettle, which is hence called the draw-off kettle [4]. The ingredients are fed into the cooking chamber by dosing or manually. The cooking temperature and time are controlled automatically. The product after cooling is discharged into the draw-off kettle through suction created by vacuum pump. During this process, the cooked mass will be evaporated under vacuum pump. The draw-off kettle is lowered after reversing the vacuum to unload the product.

37.4.1.4 Rotary Cereal Cooker

This is a single rotating pressure cooker, controlled either automatically or manually and mounted on a horizontal axis. The cooker consists of a stainless-steel barrier with a slide valve or door for charging and discharging; steam is injected at 200 kPa working pressure through a flexible hose and rotary

union [5]. The pressure cooker consists of an all-welded stainless-steel double-conical barrel with annular rings at each end to which are bolted endplates. The barrel has a centrally positioned opening fitted with a door for charging and discharging. Steam injection is through a 50-mm diameter stainless-steel braided flexible hose and rotary union mounted at the end of each stub shaft. Steam passes through the center of each shaft into a stainless-steel steam chest, which is integral with the shaft and endplate. Steam injection and exhaust are controlled through a system of stainless-steel ball valves. Pressure in the cooker is indicated at each end by a pressure gauge.

37.4.2 Continuous Cookers

37.4.2.1 Pressure Cookers/Blanchers

The Balfour continuous pressure cooker: The Balfour continuous pressure cooker can handle particulate foodstuffs ranging from cereals and diced vegetables to regular whole beetroots and potatoes, including diced meat. It is designed for the continuous feeding of process materials into and removal from the cooking vessel, which utilizes steam under pressure. The retention time of the process material can be strictly controlled and varied according to requirements [5]. The main cooking section consists of a plain helical screw conveyor operating within a circular body—the body being designed to operate at the maximum steam pressure. Pressure lock systems are fitted at both the inlet and outlet ends. The size of the cooker is directly proportional to the required cooking time and inversely proportional to the total process time [6].

The Turboflo blancher/cooker: The Turboflo hydrostatic blancher/cooker uses a unique steam injection and energy-circulation system for blanching and cooking potatoes, vegetables, fruits, meats, and poultry. It combines the benefits of energy efficiency and improved product quality and saves processing time and also requires less floor space. The cooker achieves process time savings of up to 30%. The fully enclosed and insulated chamber prevents steam from escaping and saves energy. Products blanched in this cooker retain more nutrients and have both better color and better taste and thus customer appeal. The energy-circulation method features a fan-driven steam path, which penetrates the product mass evenly to assure thorough blanching/cooking of the product. The modular section design provides maximum flexibility for food processors [7].

37.4.2.2 Steam Cookers

A continuous steam cooker has been developed at CFTRI. The equipment is a continuous conveyor with a facility for open steaming into the chamber. A water inlet is provided through a flow meter to add a measured quantity of water during processing. The chamber is steam-jacketed for additional heating. A variable-speed drive is provided to vary the residence time of cooking. A rotary valve is fixed at the inlet end to control the material feed rate. The conveyor speed and the rotary valve speed are matched with sprocket and chain drive [8]. A stationary water-draining device with a stainless-steel trough and stainless-steel and nylon sieving screens is installed at the discharge of the machine, which greatly helps in the quick separation of water from the cooked product.

The product to be cooked is carefully washed, and water is fed into the cooker at a measured rate (flow rate depending upon the output and variety). Steam is let into the system by opening the steam valves at controlled pressures. Steam supply is maintained at a constant rate for both the spreader and the jacket. The product is continuously fed from the hopper through the rotary valve at the required rate. At the end of the set residence time, a uniformly cooked product is discharged through the outlet chute. The product is collected from the mesh and is ready for consumption.

37.4.2.3 Microfilm Cookers

Microfilm cookers are available as atmospheric or vacuum cookers. They are suitable for all types of high boiling and can also be used for low boiling. They are very efficient cookers using the scraped-film principle, which results in extremely rapid cooking. This reduces process inversion to a minimum. The scraped film also means that confectionery containing milk products can be handled on this machine without the risk of burning or the need for frequent cleaning [5]. The principal uses of this machine are for feeding possible depositing food products. In the case where a depositor is to be fed, a vacuum cooker should be chosen. The plant starts with a free-standing stainless-steel reservoir for dissolved syrup. This is fitted with inlet filters. The syrup is pumped from the holding tank into the cooker by an infinitely variable syrup feed pump, which has a manually adjusted control. The preheater, rotor, syrup feed pump, discharge pump, vacuum pump, metering pumps, electrical control panel, and steam controls are all mounted on a column framework.

The rotor is a scraped film evaporator made up of a bronze steam-jacketed tube with a high-speed rotor fitted inside the center. The rotor has hinged blades, which wipe the inner surface of the tube. The sugar is spread in a very thin film and is moved through the cooking tube by a combination of gravity and the design of the hinged blades. The cooked sugar is discharged from the microfilm cooker in one of two ways, either by gravity from the base of the rotor or by a discharge pump and delivery pipe. Gravity discharge can only be used in the case of atmospheric cooking. Where vacuum cooking is used, a discharge pump is necessary to withdraw the sugar from the vacuum. A discharge pump can also be used with an atmospheric cooker.

37.5 EFFECTS OF COOKING ON NUTRIENTS

During cooking great changes take place in the nature of food. Different foods behave in different ways when cooked. These effects apply to all foods [9].

37.5.1 Fats

When fats are heated, they melt, and if they contain water, it is driven off as water vapor. At 100°C, fats containing water appear to boil; this is caused by the water being given off as

steam. Fats are stable to heat and can be heated almost to their boiling point before they start to break down. It is because of this fact—and also because they have high boiling points—that fats are used for cooking. When fats are heated too much, they break down, producing an unpleasant-smelling smoke. Fat on the outside of meat and in bacon darkens in color on strong heating, and if the temperature is too high some breakdown and charring may occur. Unsaturated fatty acids are more susceptible to oxidation than their saturated analogs, and polyunsaturated content in aquatic species was demonstrated to decrease during storage and cooking [10–13]. It was found that polyunsaturated fatty acids content remained unchanged in some kinds of products under certain methods of treatment [10, 14]. Gladyshev et al. [15] studied the fatty acids in fillets of humpback salmon unfrozen, boiled, fried, roasted, and boiled in a small amount of water. Heat treatment in general did not decrease content of polyunsaturated fatty acids of the ω-3 family (eicosapentaenoic, EPA; and docosahexaenoic, DHA), except a modest reduction during frying. Cooked humpback appeared to be a valuable source of essential ω-3. It was hypothesized that the absence of a significant reduction of polyunsaturated fatty acids in the red flesh of fishes of the salmon family during heat treatment may be due to a high level of natural antioxidants which formed in the course of evolution as adaptation to their ecological niche [15].

37.5.2 Carbohydrates

When exposed to dry heat, carbohydrates are broken down and darken in color. For example, sucrose browns on caramelization and finally chars and becomes black, while starch is broken down into more easily digested dextrin and also darkens and eventually chars. Many foods that contain both sugars and protein turn golden brown and change flavor on heating. These changes occur in the toasting of bread and the baking of bread, cakes, and biscuits, and contribute to the pleasant flavor and attractive color of these products.

When a mixture of starch and water is heated, the starch granules absorb water and swell and gelatinize, forming a thick white paste. This is why starchy material (e.g., flour) is used to thicken sauces. On cooling, the paste sets and forms a gel. Uncooked starchy foods are difficult to digest because the digestive juices cannot penetrate into the starch grains. Cooking causes the starch granules to swell and gelatinize.

The polysaccharides starch, cellulose, and pectin are important constituents of fruit and vegetables. On cooking, insoluble cellulose changes little, except to soften, whereas starch softens as it gelatinizes and pectin becomes more soluble and dissolves somewhat, allowing cells to separate and making the fruit or vegetable easier to eat. Fruit with a high pectin content, such as apples, becomes soft and pulpy on cooking.

37.5.3 Proteins

Proteins undergo great changes when they are heated. Many proteins coagulate when heated; for example, egg white coagulates when it is heated above 60°C. As proteins coagulate they become solid. For example, when milk is heated a skin forms because some of the proteins have coagulated. Cheese is another important protein food, and when it is heated it softens and on further heating, some of the proteins coagulate and the cheese becomes stringy and tough. Not all proteins coagulate on heating, which is important when considering how to cook protein foods. Collagen and elastin, for example, are two important insoluble proteins in meat, and because they are not soluble, they are not easily digested. Their presence in meat makes it tough, and as the cheaper cuts of meat usually contain more collagen and elastin than more expensive ones, they are usually tougher. Tough meat must be cooked in a way that will make it tender. If such meat is cooked at high temperatures for long periods it remains tough or may even become tougher. Tough meat needs to be cooked slowly using low temperatures; both dry-heat and moist-heat methods may be used.

Tough meat is often cooked slowly using moist heat (e.g., stewing). This converts the tough collagen into gelatin. Gelatin is a soluble protein and so is easily digested. Slow cooking using dry heat is also effective in converting collagen into gelatin. Elastin softens on cooking, but not to the same extent as collagen. Bertram et al. [9] studied the thermal denaturation of meat protein by NMR and DSC. The denaturation of myosin rods and light chains occurred at 53–58°C, and heat-induced changes in myofibrillar water as well as between actin denaturation at 80–82°C and expulsion of water from meat.

37.5.4 Mineral Elements

Heat does not affect mineral salts found in food because they are stable substances that do not break down at the temperatures used in cooking. Moist-heat methods of cooking, such as stewing and boiling, cause loss of salts, which are soluble in water. Boiled fish, for example, is rather tasteless because of the considerable loss of mineral salts that occurs during cooking. However, the salts are present in the water in which the fish has been boiled, and this liquid or stock can be used for making a tasty sauce to eat with the fish.

37.5.5 Vitamins

Dry-heat cooking methods destroy those vitamins which are unstable to heat. Vitamin C is destroyed at quite low temperatures, and so all methods of cooking cause some loss of this vitamin. To make the loss as small as possible, foods containing vitamin C should be cooked for as short a time as possible and should be eaten as soon as they are cooked. Two of the B vitamins, thiamine and riboflavin, are unstable at high temperatures. Riboflavin is the more stable of the two, and little is lost except at high cooking temperatures, such as those used in rapid grilling. Thiamine is largely destroyed at high temperatures, such as are used in grilling and roasting. Cooking with moist heat causes loss of water-soluble vitamins, as well as those that are destroyed at low temperatures. Vitamin C is

both soluble in water and unstable with heat, and therefore some loss during cooking cannot be avoided. Vitamin C is also destroyed by oxygen present in air and dissolved in cooking water. Leaching is also the main reason for high losses of ascorbic acid during cooking. The rate of destruction is hastened by enzymes present in the plant or fruit. These enzymes are set free by crushing or chopping. Different studies found a decrease of ascorbic acid by boiling up to 75% [16–18].

The B vitamins are soluble in water in varying degrees, thiamine being the most soluble. A considerable proportion of the thiamine in foods may be lost during cooking, especially if they are boiled in alkaline solutions. For this reason, alkaline substances, such as sodium bicarbonate, are not added to green vegetables to prevent loss of green color during cooking. The amounts of the other B vitamins lost during cooking are small and not important. Vitamins A and D are insoluble in water and stable except at high temperatures. There is therefore little, if any, loss of these vitamins during cooking.

In green vegetables, β-carotene is incorporated in the carotenoid-protein complexes in the chloroplasts. These carotenoproteins have an inhibitory effect on carotenoid digestion and absorption [19]. In orange or red fruits β-carotene is dissolved in oil droplets in the chromoplasts and can be readily extracted during digestion [20–22]. Food preparation, e.g., mincing and cooking, can increase the extractability and therewith the bioavailability of β-carotene from the food matrix by softening or disruption of plant cell walls and the destruction of carotenoid-protein complexes [23–26]. Cooking can also lead to an isomerization of the natural substances, mainly the all-*trans*-form of β-carotene to its *cis*-isomers. *Cis*-isomers are less bioavailable, and the provitamin A-activity is lower [27, 28]. Bernhardt and Schlich [19] studied the influence of different domestic cooking methods (boiling, stewing, steaming, pressure steaming, microwave) on the all-*trans*- and *cis*-β-carotene as well as the α-tocopherol content in fresh and frozen broccoli and red sweet pepper. In fresh broccoli all cooking methods lead to a significant release of all-*trans*-β-carotene. In frozen broccoli no change of α-tocopherol occurred. In the fresh and frozen peppers no change or a significant loss of α-tocopherol and all-*trans*- and *cis*-β-carotene was observed. A slight increase in the *cis*-isomers of β-carotene can only be found by cooking fresh broccoli. Oxidation promoted by the presence of light, heat, and oxygen is the main reason for destruction of carotenoids [29, 30].

37.6 HEALTH ASPECT OF COOKING METHODS

Polycyclic aromatic hydrocarbons (PAHs) are pollutants that are found in food products due to soil or water contamination. These are believed to have carcinogenic, cytotoxic, and mutagenic effects. PAHs are also found in charcoal-grilled or roasted food products due to the combustion of charcoal and are also found in smoked food products [31]. It has been also anticipated that PAHs are formed during frying. The smoke of oil during deep-fat frying emit PAHs, mainly three- and four-ringed PAHs [32]. Different concentrations are found in the smoke emitted from different edible oils, and that might be related to the degree of saturation of oils [33]. In 2006, a working group formed the International Agency for Research on Cancer (IARC) concluded that the emissions from the high frying temperatures are "probably carcinogenic" [34]. PAHs might be volatilized during frying [35], and this might explain the low levels of the compounds in the frying oils and samples being fried [35, 36].

The discovery of acrylamide in food in 2002 prompted concerns about the formation of this compound during cooking at high temperatures. Acrylamide is formed as a result of Maillard reactions which take place at high temperatures such as during frying, roasting, and baking. It has been considered a carcinogenic compound. The content in some commercial food products is in the range of 196–303 μg/kg [37]. Asparagine has been identified as the main amino acid involved with the reducing sugars in the formation of acrylamide [38, 39]. N-glycoside is a Maillard reaction intermediate product and is also responsible for the formation of acrylamide. Several factors have been reported to affect the concentration of acrylamide formed during frying: The frying temperature (the higher the temperature, the higher the concentration), frying time (the longer the time, the higher the concentration) [40, 41], potato variety (sugar content, mainly glucose and fructose) [5], presence of phenolic compounds in the oil [41], and pre-treatments such as blanching or soaking in acidic solutions [42]. Biedermann-Brem et al. [43] studied the effect of reducing sugar on the quality and acrylamide content of roasted potato. It was concluded that insufficient color and flavor development is obtained when the reducing sugar content is less than 0.2 g/kg (wet basis). On the other hand, a high concentration of acrylamide is formed when the reducing sugar level is greater than 1 g/kg. Acrylamide was also detected in roasted coffee beans. The temperature and roasting time affect the content of acrylamide formed [44]. It has been reported that acrylamide concentration decreases with increased roasting temperature [45–47]. Acrylamide is also formed during baking [48].

Furosine and 5-hydroxymethylfurfural (HMF) are products of Maillard reactions. They are produced during cooking methods such as boiling, grilling, roasting, and frying while frying has been shown to produce the highest concentrations of furosine and HMF. Steaming did not show any significant increase compared to the raw material [49, 50]. Different cooking methods resulted in different concentrations due to the differences in thermal damage they cause. HMF has been reported to have potential genotoxic and mutagenic activities [12, 51].

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38 Extrusion Processing of Foods

Kasiviswanathan Muthukumarappan and Gabriela John Swamy

CONTENTS

38.1 INTRODUCTION

Industrial applications of extrusion technology have led to the launch of a rich extrusion processing culture. This has developed gradually from endless communication amongst practitioners and scientists. Polymer extrusion has had the benefit of widespread technical exploration and modeling, making it promising to forecast process performances. However, food extrusion processing, along with the reported designs, is fairly new, and it suffers from a deficiency of printed material. In addition, food extrusion inclines to be more segmented and application-oriented. Therefore, a multidisciplinary approach is necessary, for which a comprehensive estimate of the process performance is not yet available. The purpose of this chapter is to provide extensive information on extrusion processing. This chapter has been inspired both by existing applications of extrusion technology and by emerging techniques. The objective of this effort is to bridge the space between long-term experience in extrusion and the science of extrusion in order to create a link between the world of extrusion practitioners and that of imaging engineers in the field of food engineering. For this purpose, the generic extrusion process idea has been applied in order to represent and converse the

extrusion processing culture and to shape the structure of the chapter content.

Extrusion is a process that combines several unit operations including mixing, cooking, kneading, shearing, shaping, and forming. Food ingredients are fed through a hopper, and the feed moves along various sections with the help of a screw and exits through a die. During the journey inside the extruder, the ingredients are formed into a dough with partial cooking. Extrusion is operated in both batch and continuous methods. This technique is extensively operated as a continuous process in the cooking of food materials, texturization, and shaping of food, impregnation of fibrous materials, and fractionation of solid–liquid media [1]. The industrial application has generated numerous foods of the modern world such as 3D snacks, snack and feed pellets, three-dimensional breakfast cereals, pellet-to-flakes cereals, partially filled cereals, crispy flatbread, puffed foods, textured vegetable protein (TVP), and encapsulated flavors [2, 3].

The food industry employs both single-screw and intermeshing corotating twin-screw extruders. In particular, the cereal and oilseed processing industry extensively uses extruders to generate products of various shapes, sizes, and textures. Extrusion technology transforms cereal flours to prepare ready-to-eat (RTE) food products and functional ingredients by a series of steps, such as kneading, cooking, forming, and texturizing functions [4]. Due to efficient technology transfer, the cereal processing industry has benefited enormously in applying extrusion technology to create value-added products. On the other hand, the oilseed processing performs initial transformation of oilseed by fractionating its main components. The resulting products include the seed hulls, vegetable oil, and proteins. Specific designs of single-screw extruders for solid–liquid separations have been designed to suit the process.

Extrusion processing is the controlled incorporation of materials and energy into a food through restructuring or reassembling operations. This thermomechanical process is very useful in producing low-fat snacks and has the advantage of increasing protein and starch digestibility, solubilizing fiber, inactivating toxins, antinutritional factors, and undesirable enzymes, such as lipoxygenases and peroxidases [5]. Starch gelatinization can occur at levels from 12 to 22% moisture content; however, it has been indicated that at low moisture contents, gelatinization is accentuated because of the high shear stress, the heat generation, and the mechanical disruption of the granules. A partial gelatinization is necessary for a further degradation that will reduce the size of sugar chains and thus the product stability after expansion will be lost. However, a lesser degradation is not enough for opening the starch granules and reducing the ability to adsorb water, which serves later as a means for expansion. For these reasons, it is important to know the microstructure changes after the extrusion process and microwave expansion of the pellets. The conversion of feed ingredients to a usable product by the application of novel and versatile techniques of extrusion can be very useful from an economic point of view. However, the microstructure and textural changes in the product after processing and during storage are still not known. The effect of extrusion temperature, moisture content, screw speed, and storage conditions on the product microstructure, texture, and functional attributes needs to be evaluated for further applications of extrusion processing.

Extrusion is rapid, flexible, and relatively inexpensive when compared to most other food processing operations. It exposes the raw material to high temperature, pressure, and shear force to mix and cause physical and chemical changes, which constitutes cooking, and gives extruded snacks a variety of shapes and flavors. Extruded snacks are frequently low in nutritional density and high in fat and starch. Twin-screw extruders, on the other hand, provide versatility and are successfully used to process complicated material systems. Snacks have long been a part of the American diet and are rapidly increasing in popularity. Extruders are the dominating technology of most snack food production. In developed countries where protein energy is deficient, snacks are widely consumed. Extrusion cooking, a dominant technology in pet food manufacturing, is a widely used technique in snacks and the confectionery industry as well.

Technological developments in the expansion of breakfast cereals and snack items, in particular, were hindered by the difficulty of achieving and then maintaining steady conditions for extrusion with single-screw extruders. A prime target of consumer advocates and nutritionists is to effectively improve the quality and nutrition of snack foods. Extruders have taken their position in the food industry, which seems to be irreplaceable. The variety of extruded products ranges from pasta, ready-to-eat breakfast cereal, and snack food, to confectionery, pet foods, and aquaculture feeds. Its most important commercial application is high-shear extrusion cooking, a process used to produce dry expanded foods in a wide range of forms. Of the many operations of an extruder, mixing, heating, and shearing are among the most important to bring about desired texture in the final product exiting from the diet. Preconditioning, extrusion, cutting, and drying are primary unit operations in most industrial extrusion processing. Extrusion of heterogeneous ingredients makes as good a use of art as it does science. Controlled expansion is a desirable feature in extrusion processing.

38.2 TYPES OF EXTRUDERS

The design of extrusion equipment, with prime focus on the hardware components, is covered in this chapter. The hardware part has been discussed extensively, as they have a direct impact on the performance of the extrusion process. Single-screw and intermeshing corotating twin-screw extruders have been elaborated, as they equally support the generic extrusion processes. Extruders can be classified as in Figure 38.1.

38.2.1 Single-Screw Extruder

Single-screw extruders are readily available in a number of shapes and sizes, and the barrel, screw configuration, and screw can be varied to suit a particular variety of product characteristics. The main advantages of single-screw over

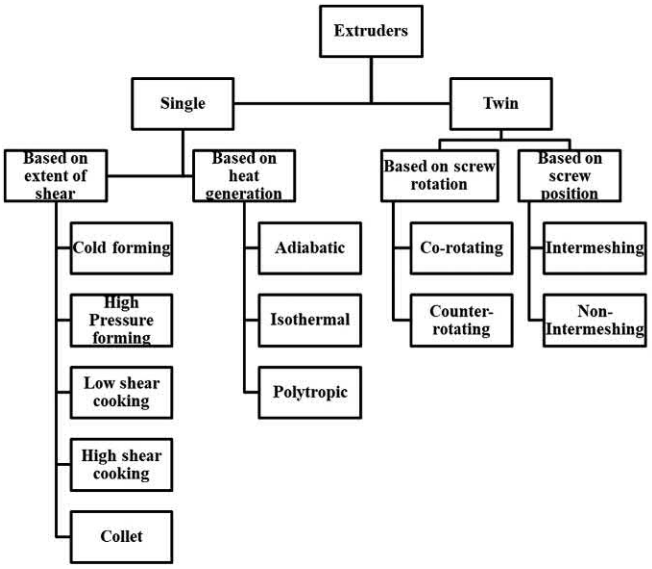

FIGURE 38.1 Extruder classification based on design and function. (From Muthukumarappan and Swamy [1].)

twin-screw extruders are that they are mechanically very simple and the cost is half the price of similar-sized twin-screw extruders. Because of this, single-screw extruders are used wherever possible in the industry and in academic research. The material is conveyed along the length of the screw by a drag flow mechanism, where drag is directly proportional to screw speed. In general, single-screw extruders possess poor mixing ability, which necessitates the premixing of ingredients prior to extrusion.

38.2.2 Twin-Screw Extruder

A twin-screw extruder comprises two screws rotating either in the same direction (corotating) or in the opposite direction (counterrotating). Based on screw configuration and degree of intermeshing, twin-screw extruders can be classified into fully intermeshing, partially intermeshing, and non-intermeshing. Corotating twin-screw extruders are the most common in the food and snack industry for their pumping efficiency, good control over residence time distribution, self-cleaning mechanism, and uniformity of processing. Counterrotating intermeshing twin-screw extruders were developed for the processing of polyvinyl chloride, which comprises resin beads that are slippery and difficult to process in a single-screw extruder. Twin-screw extruders are more flexible in operation than single-screw extruders, but they are more expensive. Some of the advantages of twin-screw extruders include the ability to handle a variety of materials (viscous, oily, sticky, and wet) and a wide range of particle sizes, a nonpulsating feed, positive pumping action, self-cleaning, and scaling up.

38.2.3 Extruder Design

The basic design of an extruder is that of a single-screw extruder. Modification in the number of screws, the direction of rotation of the screws, and the intended application are the factors that add up in the twin-screw extruder [6]. Figure 38.2 shows the design of a single-screw extruder. The functional components of extruders are classified as follows:

- Motor and the gearbox: These are responsible for the kinematics of the extruder. The mechanical power is generated by these components.
- Screw-barrel assembly: These may contain three to four sections to convert the feed to a dough-like material. A heating and cooling device may be attached depending on the product.
- Die assembly: The processed material is shaped, formed, or textured. This section is crucial, as this constriction also determines the quality of the product.

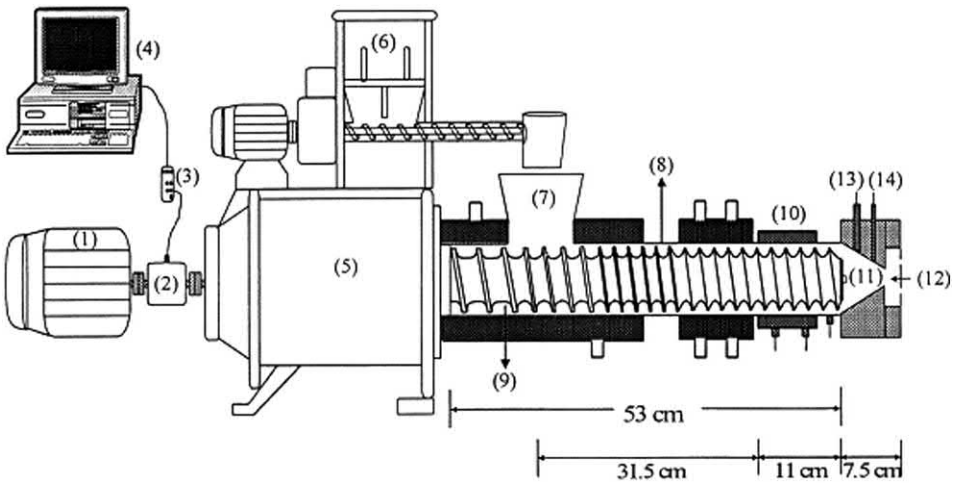

FIGURE 38.2 Single-screw extruder design: (1) motor; (2) torque transducer; (3) amplifier; (4) personal computer; (5) gear box; (6) feeder; (7) hopper; (8) barrel; (9) screw; (10) heater; (11) die plate; (12) die space; (13) thermocouple; (14) pressure gauge. (From Muthukumarappan and Swamy [1].)

- Visual and operating cabinet: The computer system helps to monitor equipment operation. The screw speed and temperature at various sections of the barrel can be set here. In addition, the resulting torque can be observed here.

38.2.3.1 Screw

The screw is the central part of an extruder. Screw diameter (D) is the distance between two flights across the screw shaft. Channel depth (h) is the distance from the top of the flight to the root. Pitch (t) is the distance between consecutive flights. All these parameters vary depending on design and the manufacturer. The helix angle (Φ) is the angle between the flight and a line perpendicular to the screw shaft, and it varies between 12° and 15°. Clearance between the flight tips and barrel (δ) is usually 0.5 mm, and it ensures efficient pumping of the material. The axial flight width (e) of a screw is usually 10% of the screw diameter. The relative motion of the screw and barrel causes drag flow, which can be calculated by applying downstream velocity over the screw channel cross-section. Drag flow is a simple function of screw speed (N) and geometry, and is independent of viscosity for any screw design:

$$Q_d = \propto N \tag{38.1}$$

where Q_d is volumetric drag flow and α is the drag flow geometry parameter. In general, geometry parameter increases with screw diameter, channel depth, and pitch. The geometry factor for rectangular channels and shallow flights can be calculated as

$$\propto = \frac{1}{2}\pi^2 D^2 h\left(1-\frac{ne}{t}\right)\sin\theta\cos\theta \tag{38.2}$$

where n is the number of flights. All extrusion processes experience heat generation from the shearing of the viscous product. The drive motor power reflects the amount of heat generated during extrusion. The total energy transfer can be approximately described using the following equation and is a product of screw speed, shear stress (viscosity, screw speed), and surface area (extruder diameter and length):

$$Z = \pi^3 D^2 N^2 \mu\left(\frac{\pi D}{2h}+\frac{ne}{\delta}\right)l \tag{38.3}$$

where μ is the viscosity of the product and l is the wetted screw length. Some manufacturers make a screw in one piece (Figure 38.3C), whereas others make them in segments (Figure 38.3A and 38.3B). These segments are interchangeable in any order depending on the requirement on the continuous spline or key of the shaft. The most frequently employed screw configurations in the food industry are variable pitch, constant depth, increasing root diameter, increasing number of flights, and decreasing diameter. In single-screw extruders, screw geometry not only influences different functions such as mixing, kneading, heat, and pressure development but also the capacity of the extruder.

The movement and transformation of material within a single-screw extruder can be categorized into three sections: feeding, transition, and metering. The feed throat of the screw accepts the low-density preconditioned or dry blend, with deep flights and long pitch facilitating movement. The function of the feed section is to ensure the transportation of material down the screw and to fill it completely. The feed section is typically 10–25% of the total length of the screw. The transition section is also called the compression or kneading section because of its function. The food material loses its powder/granular form and changes into an amorphous or plasticized dough, thereby increasing the density. A decrease in screw pitch and flight depth and angle are the most common ways to achieve transition/compression/kneading. The transition section is the longest section of the screw, being approximately half the total screw length. This section of the screw can have forward, neutral, or reverse pitched elements depending on the application. The metering section is the part of the screw with shallow flights, which increases shear rate to maximum within the screw. A twin-screw extruder has more options because the entire screw section can consist of combinations of conveying elements, kneading elements, reverse screw elements, and additional elements. A combination of thermal and mechanical energy inputs plasticizes the food material above its melt transition temperature thus increasing the density. The compression ratio (channel depth at feed to channel depth at discharge) has a direct impact on shear development and temperature profile within the extruder barrel. A gradual decrease in flight depth in the direction of discharge and a decrease in pitch in the compression section are the most common ways to achieve compression. The compression ratio typically ranges from 1:1 to 5:1. However, for excluding air from the cereal product and improving heat transfer efficiency, a modest compression of less than 3:1 is often used.

A good mixing action is one of the most important functions of an extruder. As mentioned earlier, single-screw extruders have poor mixing ability, but this can be solved by introducing a mixing section in the screw configuration. A

FIGURE 38.3 Screw design. (A) A screw element; (B) typical screw elements; (C) screw is formed by attaching several elements together in various configurations. (From Moscicki [22].)

venting screw is used whenever necessary to vent moisture or other volatile gases trapped or contained within the extruder. A hole made in the barrel at a proper position and a larger fill in the screw releases the pressure of the material.

38.2.3.2 Barrel

The barrel is the cylindrical housing that accommodates the rotating screw and should be mechanically strong enough to withstand the pressure developed in the barrel and resist wear. A common practice is to relate the barrel length to diameter (L/D) ratio with the throughput of a single-screw extruder. Barrels are composed of honed and nitrided stainless steel (416) in various L/D ratios. Nitriding may not be effective when abrasive materials are fed into the extruder. Hardening of stainless steel lowers corrosion resistance but has a negative effect on heat transfer. A thicker biometallic coating not only addresses abrasion and corrosion but also improves wear resistance. The L/D ratio can be varied to accommodate the geometrical design of the individual components. It has been observed that food extruders typically have L/D ratios ranging from 1:1 to 20:1. For macaroni extrusion, screws are designed with L/D ratios between 6:1 and 9:1 with a screw diameter of 120–200 mm. However, an L/D ratio of 30 is required for accomplishing both cooking and forming of cereals in a single extruder. Typically, twin-screw extruders have a shorter barrel length than that of single-screw extruders. It is observed that the L/D ratio has no real meaning with twin-screw extruders in the usual sense, probably because the feeding is controlled by other devices as described later. Most of the food materials are sticky in nature during cooking and thus smooth-bore extruder barrels are not desirable. In order to accomplish positive transport, a material must slip on the rotating screw and this is enhanced if the barrel wall is grooved (longitudinal or spiral). In general, the extruder barrel is segmented and these segments are jacketed to allow temperature control of individual zones. Heating is typically accomplished with overheated steam, hot oil, or band heaters, whereas cooling is achieved with tap water. Heaters are usually located along the barrel, with a thermocouple in each zone to control the heaters and barrel temperature. The most common type of thermocouple used on extruders is the K thermocouple. Cooling facilitates the handling of products after extrusion by increasing the viscosity, which results in better retention of shape, and by reducing stickiness. Low shear stress (forming) extruders are used to densify materials with high moisture content and have a long L/D ratio, which imparts a low level of mechanical energy per unit of throughput. Expanded products are produced in high shear stress extruders, which have the shortest L/D ratios.

38.2.3.3 Die

Dies are openings at the end of the die section through which the product is extruded (Figure 38.4). They play an important role in determining the physical properties of the product such as density, expansion, surface texture, and final shape based on die design, extrusion configuration, processing conditions, and blend. Highly restrictive dies increase barrel

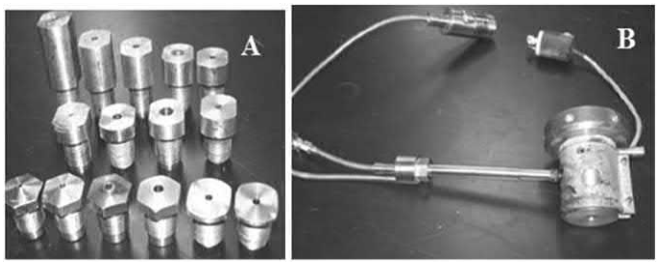

FIGURE 38.4 (A) Die nozzle designs. (B) Die design. (From Moscicki [22].)

fill, residence time, and energy input. The simplest form of die is a hole, annular openings and slits being common. In general, small-scale extruders have only one opening in the die assembly, whereas large-scale extruders have multiple openings, which alter die shear rates. Cereal processing normally requires multiple die openings. High shear rate dies are responsible for imparting energy to starch-bearing products, thereby promoting starch damage that results in an increase in water solubility in addition to other final product characteristics. Die insert, die plate, and breaker plates are other options of die used in the food industry.

38.2.3.4 Feed System

In order to achieve consistent and uniform operation of an extruder, ingredient feeding should also be uniform and consistent. Feed throat and hopper geometry should allow material to flow freely into the extruder with minimum restriction. The feed hopper should have sufficient capacity for continuous operation and it is an integral part of the feeding system. The feed rate of modern extruders is typically controlled by a variable speed auger and vibratory feeder weigh belts. Live bottoms are hoppers equipped with devices at their discharge outlets that ensure a continuous flow of ingredients; the volume of material must be sufficient to allow an orderly shutdown of the system if necessary. When the fat content of a formulation exceeds 12%, that portion of fat above the 12% level should be introduced into a single-screw extruder in a separate ingredient stream. Preconditioning is an essential step, blending steam and water with dry ingredients to achieve temperature and moisture equilibrium. This operation not only hydrates the dry ingredient but also begins the cooking under slower shear conditions than those that exist in the extruder and this process allows the extruder to focus on final heating and forming. Preconditioning increases residence time and capacity and reduces mechanical energy consumption. Preconditioning enhances flavor development and aids the final product texture. Modern preconditioners have a double-shaft design with different dimensions and speeds, and can be operated at both atmospheric pressure and elevated pressures, resulting in better mixing with retention times between 2 and 4 minutes.

38.2.3.5 Drive System

The main function of the drive system is to provide power to rotate the extruder screw(s). The drive usually consists of an

electric motor, a reduction gear, a torque transfer system, and a bearing support mechanism. The size of the motor depends on the application and capacity of the extruder and may be as large as 400 hp. For instance, a low shear stress (slow speed) extruder may require a motor of 13 hp, whereas a high shear stress extruder used for the production of light density expanded snacks may require a motor of 160 hp per ton of throughput. In general, the synchronous speed of an electric motor is 1800 rpm; however, the actual maximum speed of the motor is 1725–1750 rpm because of slip. The screw speed of food extruders is normally less than 500 rpm. Required speed reduction is usually accomplished through a V belt or gear. Gearbox construction for a single-screw extruder is simple because the gearbox has only one output shaft to drive the screw. Gearbox construction for a twin-screw extruder is complicated because there are two output shafts to rotate two screws and because there is limited radial space to accommodate bearings to support both the radial and thrust load from the extruder. In general, a three-bearing arrangement (two to support the radial load and a third to absorb thrust load) is used if a single-screw extruder has a motor size of more than 100 hp.

38.3 EXTRUDER VARIABLES

Screw speed, barrel temperature, screw and barrel configuration, die opening, and feed rate are some of the parameters that affect extruder performance. Extruder operation depends on pressure buildup in the barrel (prior to exiting the die), slip at the barrel wall (transportation), and the degree of filling.

38.3.1 Screw Speed

In general, screw speed is responsible for the rate of shear development and the mean residence time of the feed. The heat dissipation from the mechanical energy input to dough depends on screw speed, which in turn influences dough viscosity. In some cases, the completion of texture formation and chemical reactions within the barrel requires a long residence time, which corresponds to slow screw speed.

38.3.2 Barrel Temperature

In order to avoid plugging and backflow of material, the feed zone temperature is low and barrel temperature ramps up as the material travels down the screw. Barrel temperature usually has a positive effect on the degree of starch gelatinization and extrudate expansion, whereas it has a negative effect on product color, especially at elevated temperatures. Several studies have indicated that elevated temperature leads to more moisture evaporation when exiting the die, and thus results in more expanded products.

38.3.3 Feed Rate

Extruder feed rate depends on the type of screw element, screw speed, type of feeding element, and feed moisture. Feed rate has an influence on residence time, torque requirement, barrel pressure, and dough temperature.

38.4 FEED INGREDIENT VARIABLES

Feed composition, moisture content, and particle size have the greatest effects on extrusion. These factors are discussed in the following sections.

38.4.1 Feed Composition

The typical composition of any blend consists of starch, protein, lipid/fat, and fiber, which all contribute to product quality. Starch degradation usually reduces product expansion. It is essential that infant and weaning foods have high starch digestibility, which is largely dependent on full gelatinization [7]. Lipid levels over 5–6% act as a lubricant, reducing slip within the barrel and resulting in poor product expansion. If the production of porous and expanded product is not the target, then a fat level of 15–18% can be used in single-screw extruders and a fat level of 20–22% in twin-screw extruders. The lipid content of the extruded product is low. Rancidity is an issue for extruded products during storage because of lipid oxidation, which causes rapid deterioration of sensory and nutritional qualities [8]. Sugar and salt (functional ingredients) have more effects on wear than other ingredients. In cereal processing, sugar concentration has a negative effect on viscosity, and high sugar concentration inhibits gelatinization, requiring higher temperatures to achieve the same degree of product expansion. Salt will assist in obtaining uniform moisture migration after drying of third-generation pellets during moisture equilibration. In general, salt reduces water activity, which leads to poor product expansion. Generally, fiber is a noninteracting component that contributes to low expansion, cohesiveness, durability, and water stability. High fiber content usually results in high screw wear [9].

38.4.2 Feed Moisture

Moisture is a critical variable that has multiple functions in starch gelatinization, protein denaturation, barrel lubrication, and final product quality. Processing is uneconomical at in-barrel moistures below 20% and results in undesirable nutritional quality. However, a dry extruder can process materials with 8–22% moisture with no additional drying of extrudates. In general, a medium shear stress extruder can handle food with 16–30% in barrel moisture, whereas a low shear stress extruder can handle food with more than 30% in-barrel moisture (pasta dough has 31%). An increase in moisture content will have a pronounced effect on the rheological properties of the melt in the barrel. High-moisture feeds decrease the mechanical energy requirement and reduce the wear and thereby operating cost. However, most extruded snacks have a moisture content between 8% and 12%, and require additional drying to impart the desired texture and mouthfeel. High moisture reduces vitamin loss during extrusion due to limited thermal degradation.

38.4.3 Feed Particle Size

As a general rule of thumb, the extruder feed should not have particles larger than one-third the diameter of die holes. Particle size also plays an important role not only in moisture distribution, heat transfer, and viscosity but also in final product quality. Coarse ingredient particles have more effect on wear than fine particles. A product composed of fine particles will have good water stability, water absorption index, expansion, and floatability.

38.5 INTERRELATIONSHIPS OF EXTRUDER AND INGREDIENT VARIABLES

A better understanding of the interactions between the extruder and ingredients within the barrel facilitates the development of not only screw and barrel configurations for converting mechanical energy to heat through friction but also new products. Extrusion experiments typically examine two to three variables, but many factors are important. The outer square shows the primary extruder and ingredient variables and the inner square shows the secondary variables. Viscosity is a reflection of the molecular weight of functional polymers and its measurement correlates with the energy input to an extruder. Although viscosity and residence time are placed in the inner square, they are affected by other variables in the same square. In general, an extruder converts ingredients into dough. Gelatinization of starch causes a substantial uptake of moisture, resulting in an increase in dough viscosity. An increase in feed rate and screw speed and a reduced L/D ratio has a negative effect on product residence time, which in turn affects dough viscosity. As screw speed increases, dough viscosity decreases because dough exhibits non-Newtonian behavior. High temperature and low moisture are responsible for the formation of Maillard compounds and heterocyclic chemicals, resulting in a typical cooked grain flavor. High barrel temperature, screw speed, specific mechanical energy (SME), low feed moisture, die diameter, and throughput are the variables that increase vitamin loss during extrusion. A die with long land results in a dense product and thereby increased bulk density. The amount of starch, protein, and fat present in the blend affects product expansion and firmness. The torque indicates the amount of energy absorbed by the material due to shear exerted by the screw(s). Motor torque is a very sensitive indicator of stable operation during extrusion. Fluctuation in motor torque usually indicates non-steady state extrusion conditions; it occurs when there is erratic feeding and surging, and may cause plugging of the die. Therefore, one must ensure uniform and consistent feeding. SME indicates the extent of molecular breakdown or degradation that the material undergoes during the extrusion process. An increase in moisture content decreases the viscosity of dough in the extruder, shortens the mean residence time, and reduces the conversion ratio of extruder mechanical energy into heat energy, and consequently SME becomes lower.

In general, the power supplied to a screw is used to transport, compress, and shear the melt/mass. Considering shear stress on the melt at the inner wall and the contact area of a single-screw extruder, the power requirement can be calculated using the following equation:

$$P = \tau\pi^2 D^2 NL \tag{38.4}$$

where P is power (W), τ shear stress (N·mm^{-2}), D screw diameter (mm), N screw speed (rpm), and L length of filled section (mm). This equation defines the thermomechanical process for a given screw geometry. SME is defined as the mechanical energy input required to obtain the unit weight of extrudate and can be calculated by the following equation:

$$SME = \frac{\omega}{m_f} \tag{38.5}$$

where SME is specific mechanical energy (W·h·kg^{-1}), Γ torque (N·m), ω angular velocity (s^{-1}), and m_f mass flow rate of feed (kg·h^{-1}). These equations show that the torque developed in an extruder is apparently the product of shear stress and filled length since the screw speed is expressed as angular velocity. Screw speed directly influences extrusion because the peripheral velocity of the screw (πDN) is proportional to screw speed. The filled length and shear stress during extrusion depend on total throughput and in-barrel moisture content.

Typically, SME is an indication of the viscous dissipation of mechanical energy, which is provided by the screw drive shaft, into the dough due to frictional resistance. The SME for direct expansion of cereals is as high as 80 W·h·kg^{-1}, with high shear provided by high screw speeds and/or shallow screws. As screw speed increases, SME also increases because the changes in energy input to the screw are of a greater order of magnitude than the decrease in torque associated with the decrease in apparent viscosity due to the shear thinning behavior of non-Newtonian material. An increase in barrel temperature generally leads to a decrease in dough apparent viscosity, which not only results in low SME to the drive screw shaft at a given speed but also produces a more expanded product on die exit.

Any variable affecting the viscosity of the material in the extruder would correspondingly affect torque [10]. Feed composition plays a role in affecting secondary extrusion variables including torque. At low screw speeds, the residence time of the viscous melt is increased, allowing greater plugging of the die section and subsequent increases in torque. The torque required to turn the extruder screw is also related to degree of fill in the extruder barrel. Increasing barrel temperature contributes to a decrease in viscosity, which results in flowable material and thus a lower required driving force. At constant temperature, viscosity decreases with increasing moisture content because water acts as a lubricant.

Residence time influences the degree to which the raw material experiences shearing, heating, shaping, mixing, and reaction. The effect of moisture content on the mean residence time is the result of two opposing effects of moisture content on rheology of feed material in the barrel and die of the extruder. On the one hand, increasing the moisture

content of feed material results in a decrease in viscosity of feed dough in the barrel of the extruder, and lower force is required to pump the melt through the die. On the other hand, temperature in the die due to viscous dissipation is lower, and the lower temperature of the feed increases the viscosity at the die, which tends to increase the restriction of flow through the die.

38.6 FUNCTIONS OF AN EXTRUDER

Numerous functions can be carried out in an extruder for food, feed, and other industrial applications.

38.6.1 Agglomeration

Ingredients can be compacted and agglomerated into discrete pieces with an extruder. The main purpose of agglomeration is to improve certain physical properties of food powders such as bulk density, flowability, dispersability, and stability. Agglomerated products are easy to use by consumers and hence are preferred over the traditional nonagglomerated products that are usually nonflowable in nature. One of the problems identified in the pasta-making process is the formation of large wet agglomerates. Researchers have characterized the wet agglomeration properties of semolina and a whole hard white winter wheat flour enriched with flaxseed flour [11, 12]. The flour compositions were semolina 100% (S), whole wheat 100% (WW), semolina–whole wheat (49:51) (SWW), semolina–flaxseed (SCF/SFF) (90:10), whole wheat–flaxseed (WWCF/WWFF) (90:10), and semolina–whole wheat–flaxseed (SWWCF/SWWFF) (39:51:10). Samples were hydrated to 30%, 31%, 32%, 33%, and 34% moisture content and extruded. SFF hydrated above 30% moisture had the largest agglomerates. Recommendations were 30%, 32%, 33%, 34% moisture for SFF, SCF, WWFF and SWWCF.

38.6.2 Dehydration

During normal extrusion processing, a moisture loss of 4–5% can occur. The rate of moisture loss depends on the feed. In the case of high starch products, moisture loss can be greater than 80%. In certain cases, the situation depends on the feed and process parameters. A barley–oat breakfast cereal supplemented with 10% green or yellow pea was extruded at barrel temperature 81.9–103.6°C, die temperature 57.3–77.3°C, and screw speed 200 rpm [13]. The physicochemical properties of extruded cereal were determined, and it was found that the moisture content increased with increase in oat content. In other cases, the moisture flashes out as vapor and the resultant product is crispier.

38.6.3 Expansion

Product density can be optimized by extruder operating conditions and configuration. The extruded product usually puffs and changes texture due to the reduction of forces and release of moisture and heat. The extent to which it does so is known as the expansion ratio. Corn starch with carrot pomace (5, 10, and 15 g/100 g) was extruded at with initial feed moisture levels of 15, 22.5, and 30 g/100 g [14]. Corn starch without any added pomace was extruded as a control treatment. Maximum expansion was observed in extrudates with 5 g/100 g carrot pomace and at 15 g/100 g feed moisture.

38.6.4 Gelantinization

Extrusion cooking improves starch gelatinization. During extrusion, the physicochemical transformation of starch takes place without any additional chemicals. Barothermal treatment causes gelatinization of starch. This process is accompanied by rupture of intermolecular bonds, resulting in rupture of starch grains and a significant increase in water absorption. The amylose fraction of starch has greater binding properties than the amylopectin fraction. The degree of changes in starch depends on properly selected process parameters and the residence time of raw material in the extruder [15].

A twin-screw extruder was employed to blend yam, rice, and cornflour [16]. Yam flour (10–40%), feed moisture content (12–24%), and extruder barrel temperature (100–140°C) were the factors selected to characterize the gelatinization. The water absorption index, an index of starch gelatinization, varied between 5.23 and 6.34 g/g dry solid. At higher moisture content, viscosity was reduced and allowed the starch molecules to circulate. This phenomenon increased heat penetration and resulted in greater gelatinization.

38.6.5 Mixing

Various screws are accessible, which enable different mixing actions in the extruder barrel. Starch is a hydrophilic biopolymer. An intense mixing process is vital to combine the hydrophobic molecules and to attain the anticipated dispersed phase morphology. Shear stress distributions indicate that increasing screw speed decreases the maximum shear stress undergone by the particles. However, at higher screw speeds, particles are exposed to higher capillary ratios and these show that mixing efficiency is increased. In short, a decrease in the shear stresses does not essentially lower mixing efficiency because a simultaneous decrease in blend viscosity leads to a higher capillary ratio. Research indicates that increasing the screw speed not only helps fine dispersion but also retains lipophilic bioactives susceptible to mechanical stresses [17].

38.6.6 Protein Denaturation

Animal and plant protein can be denatured by extrusion cooking. The modification of proteins during extrusion cooking is mainly attributed to thermal effects and shear. Protein denaturation is the primary thermal effect. Under the influence of high temperature and moisture, native proteins lose their structure (globular, micellar), unfold, adsorb water and "melt." Just as starch gelatinization, in extrusion cooking, protein denaturation occurs at lower moisture content, resulting in a

high viscosity melt [18]. In the case of soy protein, it has been shown that the protein in the extrudate is completely denatured, provided that extrusion temperature was above 130°C. Pea and kidney bean seeds were extruded at 148°C and 156°C, respectively [19]. Protein solubility at various pH values and in various solvents was carried out. Analysis of protein fractions was performed by SDS-PAGE. Results indicated that protein solubility from both raw and extruded legumes was significantly higher in saline solutions. The protein solubility of extruded pea and kidney bean flours decreased sharply with respect to native flours when extraction was done in a buffer (pH 7.0) alone. The experiment suggests that extrusion caused insolubilization of protein by noncovalent interactions and disulfide bond formation. Denaturation and aggregation led to a decrease in the superficial hydrophobicity resulting in the establishment of a three-dimensional network with higher water-holding capacity, smaller oil absorption capacity, and lower protein solubility. Furthermore, aggregation decreased the protein solubility.

38.6.7 Texture Alteration

Textures can be changed by varying operational parameters and feed/additives in the extrusion system. The extruded product usually puffs and changes texture because of the reduction of forces and release of moisture and heat. Research was conducted to formulate a functional dairy ingredient by extruding milk protein concentrate with 80% protein [20]. The ingredient was incorporated in high-protein nutrition (HPN) bars. This resulted in favorable texture attributes, such as reduced firmness and improved cohesiveness. Protein ingredient, storage temperature, and storage time had a pronounced effect on the max force, shear force, adhesiveness, cohesiveness, and crumbliness during instrumental texture measurement. The control sample became less cohesive around week 18 at 22°C and week 10 at 32°C. During the two-bite test, after one compression the HPN bars deformed. The crumbliness of the HPN bars with extruded MPC80 increased gradually at 22°C, whereas the increase was more distinct at 32°C. Extruded MPC80 HPN bars were softer, stable, and cohesive as compared to spray-dried control MPC80 even after extended storage.

38.6.8 Thermal Cooking

Instead of just combining feed, a desired cooking effect can be achieved in the extruder. The cooking process takes place within the extruder where the product produces its own friction and heat due to the pressure generated (10–20 bar). The process can induce both protein denaturation and starch gelatinization under some conditions. A ready-to-eat snack was developed from finger millet through extrusion cooking. Seven composite mixes were formulated with different compositions of brown finger millet flour, maize flour, rice flour, full-fat soy flour, Bengal gram flour, and skimmed milk powder [21]. Extrusion cooking was carried out using a twin-screw extruder. The operating parameters were temperature 140°C, screw speed 300 r min^{-1}, and die diameter 3 mm. The bulk density ranged from 0.1618 to 0.3946 g cm^{-3}, and expansion ratio values oscillated between 2.42 and 3.50. The sample containing the least amount of finger millet flour of about 10% had the lightest color, while the mix with the highest amount of brown finger millet flour (40%) had the least value for hardness. The average scores of sensory evaluation indicated that all the extruded products were within the acceptable range. The most acceptable extrudates were obtained from brown finger millet flour, maize flour, rice flour, and full-fat soy flour in the ratio of 20:50:20:10, which had an expansion ratio of 3.5, hardness 23.37 N, and organoleptic characteristics 8.87.

38.7 ADVANTAGES OF EXTRUSION

The principal advantages of extrusion technology compared to traditional food processing methods include the following:

- *Adaptability*: A wide variety of extrudate production is feasible by altering the minor ingredients and the operational parameters of the extruder. In addition, it can enhance product differentiation by accommodating consumer demand.
- *Product characteristics*: The shape, texture, color, and appearance can be changed very easily without major changes in the hardware, which is not easily feasible using other production methods.
- *Energy efficiency*: Redrying of extrudates is not required, as the extruders operate with relatively low moisture while cooking food products.
- *Low cost*: The estimated savings using the extrusion process are raw material (19%), labor (14%), and capital investment (44%). Moreover, it needs less space per unit of operation as compared to traditional cooking systems.
- *New product line*: Extrusion has the ability to transform animal and vegetable proteins, starch, and other food materials to create unique and attractive food products.
- *High productivity and automated control*: An extruder delivers continuous high-throughput processing. It is completely automatic.
- *High product quality*: Extrusion is a high-temperature/short-time (HT/ST) heating process. This reduces nutrient loss and improves the digestibility of protein and starch. In addition, high temperatures also destroy antinutritional compounds such as trypsin inhibitors, detrimental enzymes (lipases, lipoxidases), and microorganisms.
- *No effluent*: New environmental regulations are stringent and expensive. Extrusion results in little or no waste streams.
- *Process scale-up*: Pilot plant experimental results can be used to scale up the extrusion system for industrial production.
- *Use as a continuous reactor*: Extruders are being used as continuous reactors in several countries

for deactivation of aflatoxin in peanut meals and destruction of allergens and toxic compounds in castor seed meal and other oilseed crops.

38.8 CRITICAL FACTORS IN EXTRUSION PROCESSING AFFECTING MICROSTRUCTURE

Raw ingredient compositions, processing equipment selection, and processing parameters are independent variables that can be experimented to create new extrudates [22]. Though the parameters are independent, they are correlated since discussions on one variable will usually encompass the others. Selection of raw materials has a remarkable influence on the extrudate texture, uniformity, extrudability, nutritional quality, and economic viability. Generally, extrusion converts cereal/pulse blends into a dough. The starch gelatinizes and increases moisture absorption, thereby increasing dough viscosity. On the other hand, protein influences the elasticity and gas-holding properties, which is a characteristic of hydrated and developed glutinous doughs. Some proteins are responsible for the adhesive and stretchable functional properties.

The rich nutritional property is the basis for raw material selection in extrusion. Economics and raw material availability are vital factors too. During purchase, desirable characteristics are listed and can be verified by proximate analysis. However, the nutritional properties are only vaguely recognized and no test exists to monitor quality. Storage and preprocessing prior to extrusion significantly affect when the materials are subjected to heat, pressure, and shear. Cereals and pulses that have been recently harvested behave much differently than raw materials stored for 6 months during extrusion. A sample library listing acceptable and unacceptable ingredients helps in maintaining a smooth-running extruder.

38.8.1 Selection of Hardware Components

Choosing the proper extruder configuration is critical to successful extrusion. The manufacturer of the extrusion equipment should be able to assist in tailoring configurations for processing a specific product. There are many types of extruders, and each has a specific range of applications. An improper extruder selection for the specific application very rarely results in a smooth-running process. Once the proper extruder is selected, it must be assembled correctly and then adequately maintained. Training is important, and the supplier of extrusion equipment must be able and willing to provide this service. Knowledge of what parts will wear and what their useful life will be will avoid costly and inconvenient shutdowns. Records are imperative in this endeavor and greatly reduce the necessity of costly parts inventories. Processing equipment selection is highly important. The highest operating efficiency and versatility at low cost are the key factors considered during hardware selection.

The rate/capacity of the plant is crucial, as it may help in futuristic activities such as plant expansion. The extrusion system, whether a single-screw or co-rotating twin-screw configuration, must accomplish a number of phenomenon in a very short time under controlled, continuous, steady-state conditions. These phenomena include tempering, feeding, mixing, cooking, cooling, and shaping. The pressure, temperature, moisture, microstructure, texture, and resulting viscosity of the extrudate are affected by the system configuration and processing conditions. Selection of the proper system configuration includes choosing from the following hardware components: (i) feed delivery system, (ii) tempering or preconditioning phase, (iii) extruder barrel components, and (iv) die and knife configurations.

Familiarity with the extrusion properties of ingredients and the interaction of such equipment parameters as screw speed and hardware design can allow a general classification of all extruders into three categories (Figure 38.5). These three categories rank extruders by relative shear stress and product categories. Once the hardware is selected, a list of independent variables that the extruder operator can directly manipulate include

- Incoming recipe (the actual recipe, particle size, and moisture and temperature resulting from preconditioning)
- Rate (the rate at which the recipe is introduced into the extruder)
- Percent steam addition (steam at 6–9 bar can be injected directly into the material in the extruder barrel)
- Percent water addition (water at various temperatures and 3 bar can be injected directly into the material in the extruder barrel)
- Percent liquid addition (other liquids and/or gasses can also be introduced into the extruder barrel)
- Extruder and die configuration
- Temperature and flow rate of thermal fluid to barrel jackets
- Extruder speed (requires a variable speed drive)

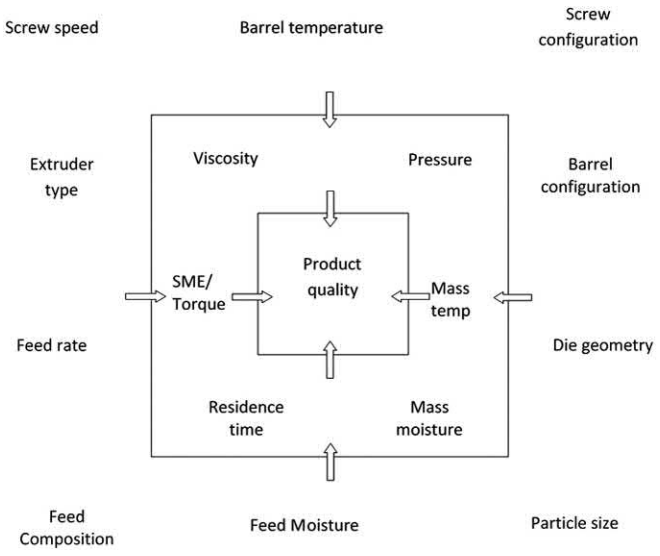

FIGURE 38.5 Interactions between extruder and ingredient variables.

When changes from this list are made, they will in turn affect other operating variables (referred to as dependent variables): (i) material retention time in the extruder barrel, (ii) product temperature in the extruder barrel, (iii) product moisture in the extruder barrel, and (iv) pressure in the extruder barrel.

38.9 SUPERCRITICAL FLUID EXTRUSION

The formation of carbon dioxide (CO_2) during extrusion normally enhances extrudate expansion [23]. During cereal extrusion, sodium bicarbonate reacts with acidulants or acids produced by the oxidation of starch during extrusion to form CO_2 [24]. Instead of relying on CO_2 formed during extrusion, CO_2 can be injected into the extruder; thus the degree of expansion and structural homogeneity of starch or cereal flour extrudates can be controlled using blowing agents. CO_2 is the solvent of choice for use in supercritical fluid extrusion (SCFX) because it is generally recognized as safe (GRAS), nonflammable, noncorrosive, and inexpensive; its critical point is at 31.06°C and 7.386 MPa. High temperatures (130–170°C) and shear used in traditional extrusion prevents the use of thermally labile/heat-sensitive ingredients such as some flavors, colors, and whey proteins. SCFX uses supercritical CO_2 (SC-CO_2) as a blowing agent to facilitate the formation of cellular structure in extrudates in place of the expansion of water upon exit of the extruder barrel in conventional extrusion. A four-step process has been developed: (i) development of a dough with gas-holding properties by mixing alone; (ii) injection of SC-CO_2; (iii) creation of controlled thermodynamic instability by manipulation of pressure and/or temperature in the extruder; and (iv) control of the degree of cell growth during setting of the product through appropriate die selection and postextrusion drying and cooling processes.

Product expansion by CO_2 offers several advantages over steam expansion: a closed-cell structure; CO_2 does not condense, resulting in cell collapse; and the product's interior is very nearly oxygen-free. Moreover, the melt pH is low because dissolved SC-CO_2 inhibits the Maillard reaction, which otherwise would cause further loss of essential amino acids. The physical properties of starch-based SCFX extrudates are governed by both extrusion and postextrusion parameters, including die geometry, pressure drop rate, residence time, ingredient composition, and drying temperature. Expansion and cellular characterization of the starch-based extrudates produced with SC-CO_2 injection may be governed by viscosity-dependent parameters, including gas retention capability, CO_2 diffusivity, and the pressure drop rate. An increase in the degree of gelatinization of starch–water mixtures using 0.45 wt% SC-CO_2 injection increased expansion and average cell density, and decreased average cell size. SCFX technology is a patented process that has already resulted in new developments in cereals, confectioneries, pastas, flavorings, pharmaceuticals, snacks, and other products. SC-CO_2 injection rate and product temperature at the die are the critical factors controlling the expansion and texture of the final product.

38.10 COST ECONOMICS OF EXTRUSION

Cost economics is essential in preparing the capital and operating cost estimates and profitability analysis of any food processing plant [25]. Based on the process flowchart, one can calculate the material and energy balances and the utility requirements of the processing plant [26]. The capital cost includes the cost of equipment, installation, commissioning, site development, buildings, and land. The cost of the extruder depends on the type of extruder (single/twin), construction material, capacity, accessories (preconditioner, dryer), and the manufacturer. Extruders are available for research and development at laboratory scales (4.5–36 $kg{\cdot}h^{-1}$) and for large-scale industrial production. A pilot-scale single-screw extruder (76.2 mm diameter and 305 mm length) with a capacity of 90 $kg{\cdot}h^{-1}$ would cost about $70,000, with an extra $20,000 if a live bottom hopper is included [22]. Capacity varies from 25 to 25000 $kg{\cdot}h^{-1}$, with motor power varying correspondingly from 22 to 450 kW. Similarly, cost of the extruder also varies from $75,000 to $750,000. The cost of a single-screw extruder is about half the price of a twin-screw extruder, and maintenance costs are lower. Although wet extruders (steam/water can be injected into the barrel) have higher capital costs than dry extruders (no external heating through addition of steam/water or jacket heating), they usually have lower operating costs. In general, wet extruders have higher capacities than dry extruders because of the large drive motor requirements per unit throughput on dry extruders.

Direct production costs can be divided into raw materials (basic ingredients, sugar, salt, color, additives, etc.), labor (operator, supervisor, plant engineer, etc.), utilities (gas, steam, water, and electricity), packaging, spares, and maintenance [27]. Fixed charges comprise depreciation of equipment and facilities, interest on borrowed capital, local tax, insurance, rent, etc. Raw material constitutes 35–60% of the cost, labor 5–10%, packaging 25–50%, utilities 5–10%, and all others including maintenance 5% [28]. The wide variation in raw material and packaging costs depends on the type of product and packaging. Packaging costs have increased considerably and depend on the type of product and size of the pack. Profitability depends on the volume of product; for instance, a high-volume product may have a profit of about 25%.

38.11 CONCLUSION

Extrusion technology has substantially modified the technological environment of processing industries in recent decades. Since its first introduction in the 1930s in the polymer industry, extrusion processing has never stopped expanding its technical contribution, owing to the establishment of extrusion cooking. Hence, extrusion processing technology is able to provide relevant and cost-effective processing solutions in the food and feed industry. The productivity, versatility, and reliability of extrusion cooking have made it one of the favorites, and currently, it is widely used in the cereal processing industry, both in food and feed areas, particularly when viscous biopolymeric materials, mixing of solids or liquid ingredients, or

special texturization are involved. Major commercial applications have been introduced over the years, which have required significant engineering development to design extrusion processing lines adapted to the specificities of extrusion-cooking technology. The complexity of food extrusion processing has clearly emerged from the different topics presented in this chapter. The complexity results mainly from the nature of the raw materials used in extrusion cooking, the specificities of the conversion process, and the physical characteristics of the final products produced by the process. Food extrusion deals with natural food materials such as starch and protein-based materials with characteristics that vary extensively depending on many factors such as botanical origin, climate, harvesting, and storage conditions. All these factors significantly affect the physicochemical ability of food materials to sustain the extrusion process. The future of extrusion processing technology will be more driven by the development of process intensification, owing to principles of sustainable development and the subsequent need for sustainable processing technologies. The need for sustainability will promote and boost the development of extrusion processing technology.

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39 Food Preservation by Freezing

Mohammad Shafiur Rahman and Jorge Fernando Velez-Ruiz

CONTENTS

39.1 MODE OF PRESERVING ACTION

Freezing provides a significant extended shelf life, and it is successfully employed for the long-term preservation of many foods. The freezing method is still one of the most widely used methods of food preservation even though several new technologies, such as high pressure, infrared irradiation, pulsed electric field, and ultrasound freezing, continue to emerge. Freezing changes the physical state of a substance by changing water into ice when energy is removed in the form of cooling below freezing temperature. Usually the temperature is further reduced to storage level (e.g. –18°C). The

freezing process can be clearly shown by using freezing or cooling curves and phase diagrams.

The freezing of foods slows down, but does not stop, the physicochemical and biochemical reactions that govern the deterioration of foods [1]. There is a slow progressive change in organoleptic quality during storage, which does not become objectionable for some time [2]. The loss of quality of frozen foods depends primarily on storage temperature, the length of storage time, and the thawing procedure. Microbial growth is usually stopped below –7°C, and both enzymatic and non-enzymatic changes continue at much slower rates during frozen storage [3].

The freezing process reduces the random motion and rearrangement of molecules in the matrix [3–5]. Freezing involves the use of low temperatures, and reactions take place at slower rates as the temperature is reduced. The presence of ice and an increase in solute concentration can have significant effects on the reactions and state of the matrix [6]. The final influence of temperature on chemical reactions due to freezing could be grouped as (i) normal stability (a temperature decrease results in a slower reaction rate, thus better stability when foods are stored), (ii) neutral stability (the temperature has no influence on the reaction rate), or (iii) reversed stability (a temperature decrease results in an increased reaction rate) [7]. Regardless of the type of aqueous system, concentration during freezing causes the unfrozen portion to undergo marked changes in such physicochemical properties as ionic strength, pH, viscosity, water activity, surface and interfacial tension, and oxidation-reduction potential. It is important to note that oxygen is almost totally expelled from ice crystals as they are formed [8]. Three types of cell damage occur due to freezing: osmotic damage, solute-induced damage, and structural damage [6].

In slow cooling, ice forms slowly in the external cells. If there is sufficient time, water from the cells migrates out by osmotic pressure. This results in cell shrinkage and some membrane damage. This water does not return to the cells on thawing due to the damage of cell wall, and the consequence is drip loss [6]. The concentration of the solute increases as freezing progresses. Thus, high solute concentrations in the unfrozen matrix, in particular high salt, can cause damage to many polymeric cell components and may kill the cell [9]. This concentration effect is present whether freezing is fast or slow. Cryoprotectants, such as sugars, are usually added to the aqueous phase to reduce salt-induced damage [6]. In addition to the concentration effect, the formation of ice within the cell may cause damage to the delicate organelle and membrane structure of the cell. As a consequence, enzyme systems may be released, leading to a variety of effects, including off-flavor production. This can be prevented by blanching, a pre-freezing heat treatment that denatures enzymes [6].

It is possible to design and control a convenient freezing process through an awareness of the mechanisms of damage for each particular food item [6]. In general, freezing preservation is far from perfect, and awareness of this fact is needed if techniques are to be developed to overcome known shortcomings and to assure that this method remains competitive with the other major methods of food preservation [10]. A strategic quality approach (quality enhancement) may provide a higher success rate for new frozen food products [11]. Product quality improvement and reduced energy or increased process efficiency are major issues related to the freezing process.

39.2 FREEZING RATE AND QUALITY

Controlling the freezing process, including careful pre-freezing preparation and post-freezing storage of the product, is an important aspect of achieving a high-quality product [12]. An important factor in the quality of frozen foods is the freezing rate [13]. Generally, fast freezing produces better-quality frozen food than slow freezing, although the reason for this is not as well-understood as is sometimes stated. The rate of freezing of plant tissue is important because it determines the size of the ice crystals, cell dehydration, and damage to the cell walls. Ice crystal structure is crucial for the preservation of the quality of frozen products [14].

In the case of animal tissue, the concentration of salt within the cells is higher than that in the extracellular region. Consequently, freezing starts outside the cells due to the freezing point depression induced by the solute concentration in the cells. As soon as ice appears, the solute concentration rises. This is a characteristic phenomenon of freeze concentration. At some point, osmotic pressure difference causes water to flow through the semipermeable cells to the extracellular region in order to balance the chemical potentials. This dehydration of the cell is accompanied by shrinkage of the cell, which is not normally lethal. The freezing rate affects this process because rapid freezing results in less cell dehydration (since water has less time to diffuse out of the cell), less breakage of cell walls, and less textural damage. The more rapid the crystallization, the smaller the ice crystals and the less damage is caused by the process of freeze concentration. Consequently, a reverse situation holds for thawing. Slow heating allows equilibrium to be reached as the melted water diffuses back through the cell wall.

In the case of plant tissue, there is evidence that large ice crystals can cause mechanical damage to cell walls in addition to cell dehydration. In agarose gels, large ice crystals (100–300 μm) with increasing interstitial spaces grow under slow freezing conditions at –25°C, while small ice crystals (1–2 μm) form during rapid freezing in liquid nitrogen [15]. Bevilacqua et al. [16] measured the diameter of the intracellular dendrites and extracellular ice crystals for meat frozen under simulated conditions similar to industrial freezing. They correlate the ice crystal diameter with the characteristic freezing time. De Kock et al. [17] studied the effect of freezing rate (cryogenic, fast; mechanical, slow) on the quality of cellular and non-cellular precooked starchy foods. Quality was determined immediately after freezing, as well as after frozen storage, by chemical, physical, microscopic, and sensory methods. The rapid freezing of cellular starchy food resulted in a better-quality product than slow freezing immediately after freezing. Rapid freezing of non-cellular starchy food, however, produced a product that was only slightly better in quality than the slowly frozen product. After storage, the

rapidly frozen cellular product was better in quality than the slowly frozen product, although the difference was smaller. The slight advantage gained by rapid freezing of the non-cellular product was lost in the progress of storage [17].

Symons [18] expressed that undue emphasis on the importance of freezing speed is sometimes misleading. Unless freezing is excessively slow, days or weeks rather than hours, most products are comparatively insensitive to the speed of freezing. In any case, an increase in volume of around 10% is associated with freezing of most foods. Broadly speaking, faster freezing is marginally better than slower in most products. This is particularly true for fruit and vegetable products, but less so for animal tissue [18]. Moreover, the initial advantage obtained by fast freezing is lost during storage due to re-crystallization [17]. Although fast freezing has advantages, some products may crack or even shatter if exposed directly to extremely low temperatures for a long period. Hung and Kim [19] reviewed the fundamental aspects of freeze cracking and strategies for its prevention. The mechanisms to explain freeze cracking vary, and the proposed mechanisms are:

Volume expansion: The volume expansion due to the formation of ice, and amount of empty space in a microstructure are the primary factors affecting the degree of mechanical damage to cells during freezing. In addition, differences in moisture content, composition, or amount of un-freezable water may cause different degrees of cracks [20].

Contraction and expansion: Cracking may also act to relieve the product of internal stress from non-uniform contraction during rapid cooling [21, 22]. On the other hand, both contraction and expansion may cause freeze cracking [23].

Internal stress: Fast freezing will cause crust formation at the surface, which serves as a shell and prevents further volume expansion when internal portion of the unfrozen material undergoes freezing. This process then contributes to the internal stress buildup later in the freezing process. Freeze cracking occurs if the internal stress exceeds the strength of the exterior frozen material during processing [24]. The distribution of the stress is the controlling factor, and it is governed by absorbing (dissipating) the stress into the structure or reflecting the stress to cause a buildup of internal stress [25].

Miles and Morley [26] studied the internal pressures and tensions in meat during freezing, frozen storage, and thawing. A maximum stress of almost 6000 kPa is possible. They found that during freezing internal compression developed at a rate that increased as freezing progressed, and most of the pressure was developed after the center had started to freeze. Generally, the circumferential tension in the outer surface of the muscle reached a breaking point, and a shallow crack formed along the length of the muscle or the surface yielded, causing a bulge [26]. Kim and Hung [25] found that size, moisture content, density, modulus of elasticity, Poisson's ratio, and porosity all had significant influence on freeze cracking. However, no single property completely explained the development of freeze cracking [25]. Excessive freezing speeds can ruin a food product. The build-up of internal pressure during very rapid freezing shatters the already frozen external layers and produces very small crystals, leading to scattering of incident light [18]. The current practice of quick freezing is generally chosen in order to save processing time (cost) and factory space. Moreover, rapidly chilled muscles become tough on freezing and thawing, a phenomenon known as cold shortening (not a problem in poultry) [18].

In foods containing microbial cultures, it is important to maintain their activity. Rapid freezing causes detrimental effects on the yeast activity of frozen dough. This may be due to the formation of intracellular ice crystals invariably lethal to yeast cell membranes [27]. Yeast activity decreased significantly when the rate of cooling was increased from 0.98 to 1.57°C per minute [28].

39.3 MICROBIAL ASPECTS

The detrimental effects of freezing on microorganisms may be desirable or undesirable, depending on the types of food products. In frozen foods, without any added beneficial cultures, microbial growth or spoilage is not desirable, whereas care must be taken to reduce the damage in cells during freezing of foods containing microbial culture. The maximum recommended storage temperature at which microbiological spoilage ceases lies between –9 and –12°C. Although microbiological spoilage can be avoided at these temperatures, the enzymes present in the product still play a part in spoilage. Hence, hygienic conditions or heat processing (blanched or cooked) can lead to a longer shelf life [18]. Freezing causes the apparent death of 10–60% of the viable microbe population, and this proportion gradually increases during frozen storage [29].

39.3.1 Pathogens and Spoilage Microorganisms

Microorganisms differ considerably in their sensitivity to freezing; thus the main concern is organisms that are likely to survive the freezing treatment and grow when the product is thawed. There is considerable variation in the ability of bacteria to resist damage by freezing [30]. In general, Gram-negative bacteria are less resistant to freezing death than are Gram-positive bacteria. Nonsporulating rods and spherical bacteria are the most resistant, while bacterial spores, such as *Clostridium* and *Bacillus*, remain unaffected by freezing. Bacteria in the stationary phase are more resistant than those in log phase [2, 29–31]. Genera commonly encountered in frozen food include *Pseudomonas*, *Achromobacter*, *Flavobacter*, *Micrococcus*, *Lactobacillus*, *Corynebacterium*-like catalase-positive rods, *Enterococcus*, *Streptococcus*, *S. lactis*-like streptococci, *Aerococcus*, and *Pediococcus* [29, 32]. Gianfranceschi and Aureli [1] studied the survival of *Listeria monocytogenes* during freezing (–50°C) and frozen storage (–18°C) when inoculated into chicken breast, ground beef, spinach, mozzarella cheese, and codfish. They observed only a slight decrease in the viable population ranging from 0.1 to 1.6 log after 57 min at –50°C. *Listeria monocytogenes* cells were more resistant to death and injury when they were inoculated in ground beef and chicken breast, whereas they were less resistant in fish. A further reduction in viability of

survival cells (up to 1.0 log) was detected after 240–300 days of storage at –18°C [1].

The effects of freezing on several foodborne pathogens were reviewed by El-Kest and Marth [33]. The modes of damage to the bacteria cells were reviewed by Archer et al. [30]; the principal site of damage to bacterial cells during freezing has been shown to be the membrane. Very rapid cooling of cells from room temperature to –150°C resulted in more lipid crystallization before any rearrangement of intramembrane particles could occur. This leads to damage being mainly limited to the area around the cytoplasmic membrane. In slowly frozen samples, phase separation of the outer and cytoplasmic membranes was induced, causing the outer membrane to be split off by extracellular ice crystal formation. This damage could be reduced by the addition of a cryoprotectant, which modifies ice crystal formation. Damage to membranes leads to the leakage of internal cell materials, such as potassium ions, β-galactosidase, low molecular solutes, amino acids, RNA, and single- and double-strand DNA. The release of these substances has been correlated negatively with cell viability [30]. Another type of damage is osmotic dehydration of the cell caused by extracellular ice formation and the resulting increase in extracellular solute concentration. This process causes the intracellular macromolecules to move close to the membranes. The development of repulsive forces gives rise to large anisotropic stresses in the membranes, resulting in deformation, phase separation, and formation of a non-lamellar phase. Moreover, salt addition and lowered pH also play a role in the complex nature of freeze injury and cell survival [30, 34].

39.3.2 Beneficial Microorganisms

In fermented foods, such as yogurt, frozen storage should increase the viability of beneficial cultures incorporated for their potential health benefits and control of other spoiling microorganisms. The potential beneficial roles of bifidobacteria in the human intestine reported include antagonistic effects on enteropathogenic bacteria, breakdown of carcinogenic N-nitrosamines, and suppression of liver tumorigenesis [35]. Thus, bifidobacteria is incorporated in dairy products. Kebary [35] studied the visibility of *Bifidobacterium bifidum* in the fermented dairy product zabady. The numbers of bifidobacteria surviving after 5 weeks of frozen storage (–25°C) of zabady was higher ($>10^7$) than the minimum level necessary to achieve the beneficial effect of bifidobacteria (10^5–10^6/mL). The total bacterial count decreased as the amount of added bifidobacteria increased. This could be due to the effect of antimicrobial substances produced by bifidobacteria [35]. In eight strains of *Lactobacillus acidophilus*, higher rates and greater activity were always obtained by storing cultures at –80°C, but most strains stored at –30°C also survived well. The viability of frozen cultures was affected more by storage temperature than by cooling rate [36]. The plate counts decreased less than 1 log cycle and fermentation activity was 40–70% when cultures of *Lactobacillus delbruchii* ssp. *bulgaricus* were stored at –80°C for 1 year. However, fermentation activity was less than 10% when cultures were stored for 1 year at –30°C [37]. The fermentation activity of *Streptococcus salavarius* ssp. *thermophilus* was similarly reduced to 10–60% after 1 year of storage at –30°C. Protective solutes can be used to improve survival.

Yeast cells in bakery products do not withstand freezing well. This can be partially compensated for by increasing the amount of yeast used in the formulation or adding improved yeast strains having a better survival rate in freezing [18]. The freeze–thaw tolerant yeast should have high trehalose content in addition to reduced activity [38]. Trehalose has been reported to perform a cryoprotectant function in the yeast cell [39]. Although the amount of yeast in the formulation could be increased, much higher levels of yeast, 6–8%, have a detrimental effect on the aroma and flavor of the baked product [40]. When dough pieces were made up and frozen immediately after mixing, yeast activity remained stable after prolonged storage periods. When fermentation was allowed to proceed after mixing and before freezing, the yeast became less tolerant of freezing temperature and its activity declined. This may be due to a change in yeast cells' membrane sensitivity [41].

39.4 PHYSICAL CHANGES AND QUALITY

39.4.1 Free and Bound Water

Different types of water are present in frozen foods. These can be broadly categorized as free and un-freezable water, which does not freeze even at very low temperatures. A major cause of product degradation is the amount of unfrozen water present in the frozen matrix. Unfrozen water is known to be reactive, particularly during frozen storage, rendering the product susceptible to deteriorative and enzymatic reactions and limiting its frozen shelf life. Thus, the concept of the glassy state is being applied to frozen foods stability, since molecular mobility reduces the reaction rates of the unfrozen water matrix and other components [12]. Generally, un-freezable water molecules in aqueous solution are immobilized translationally or rotationally by solutes [42]. The amount of un-freezable water can be measured experimentally and mathematically computed for different types of foods.

39.4.2 Weight Loss

Dehydration or weight loss should be regarded as an important quality parameter for frozen unpacked foods, mainly in animal tissue. Foods lose moisture during the freezing process because their surface is exposed to heat and a moisture gradient exists with the environment [43]. Weight loss of meat during freezing and frozen storage was found to be 0.28–2.98% during the freezing process, meanwhile the loss during freezing and storage ranged between 1.67 and 6.15% [43].

39.4.3 Recrystallization

Ice recrystallization during frozen storage influences product quality in different ways. Recrystallization of solutes and

ice in frozen foods is also important to quality and shelf life. A polymer is least prone to crystallization at temperatures below glass. In general, the rate of recrystallization is highest at the midpoint between the melting and glass transition temperatures. Fluctuations in product temperature of 2–3°C, as are likely to be found in bulk cold stores kept at –18°C or colder, are unlikely to cause perceptible damage even over long periods [18]. On the other hand, frequent large fluctuations during retail display and during the carry-home period cause ice crystals to ripen or grow, coalesce, and move to the product surface. This leads ultimately to a dried product if packaging is permeable to moisture, allowing the sublimed or evaporated water vapor to escape. The loss of moisture results in toughening of animal tissue and greater exposure to any oxygen present.

39.4.4 Retrogradation

Quality loss by staling and starch retrogradation occurs most rapidly at chill temperatures in baked goods. After baking, starch from the loaf progressively crystallizes and moisture is lost. Until a critical point of moisture loss is reached, freshness can be restored by heating and reabsorbing starch crystals. A tight wrap helps to keep the moisture content high for a certain amount of time. The complete crystallization of starch produces the crumbly texture of stale bread. Slow freezing is to be avoided in order to reduce the time spent at chill temperatures. Amylase is a useful antidote to bread staling. In general, moisture migration during frozen storage is the principal cause of staling [18, 44]. It has been reported that repeated freezing and thawing treatments favored starchy paste retrogradation, which is related to a mild hydrolysis of starch chains [45].

39.4.5 Protein Denaturation

Some protein denaturation and solubility changes are known to occur as a result of freezing, but the practical significance of these changes is not clear [2]. Fish muscle has a unique arrangement of muscle fibers. It is divided into a number of segments called myotomes, which are separated from one another by a thin sheath of connective tissue called the myocomma or myoseptum [46]. Fish deterioration during frozen storage is associated with a decrease of protein solubility and extractability, diminishing the nutritional value [47]. Quality loss during cold storage of meat is characterized by an increasing loss of water-holding capacity, a decrease in protein extractability, a decrease in sulfhydryl groups, and a slight loss in ATPase activity [2, 48]. In frozen meat, water losses are related to the denaturation of myofibrillar protein [49]. The water-holding capacity of the meat and biochemical properties of actomyosin, such as enzymic activity, viscosity, and surface hydrophobicity, are affected by freezing. The expressible moisture in adductor muscles increased during freezing and frozen storage. These changes were accompanied by actomyosin denaturation. The myosin and paramyosin of the actomyosin complex were most affected [50]. An amino group from some essential amino acids, such as lysine, can react with the carbonyl group of reducing sugars during processing or storage [51]. Peptides and amino acids are also increased in the drip fluid, as are nucleic acids, indicating protein changes and structural cellular damage [2]. During freezing water molecules freeze out and migrate to form ice crystals, resulting in the disruption of the organized H-bonding system that stabilizes the protein structure, and the hydrophobic as well as the hydrophilic regions of protein molecules become exposed to a new environment. This may allow the formation of intermolecular cross-linkages either within a protein molecule or between two adjacent molecules [46].

39.4.6 Freezer Burn

Moisture loss by evaporation from the localized surface of a product leads to freezer burn, an unsightly, white color, which can be mistaken for mold but is resolved on rehydration during cooking unless it is severe [18]. It is usually in the form of patches of light-colored tissues, produced by evaporation of water, which leaves air pockets between meat fibers [52]. Dehydration of product or freezer burn may occur when freezing an unpackaged food item in blast freezers unless the velocity of air is kept to about 2.5 m/s and the period of exposure to air is controlled. This dehydration can be controlled by humidification, lowering of storage temperatures, or better packaging [44]. A single package of spinach and cauliflower experienced a 1.5-fold increase in loss of moisture per 2.8°C rise in temperature between –17.8 to –6.7°C [53]. The loss of moisture occurs faster when product is held in a temperature cycle as compared to a constant temperature during frozen storage [54].

39.4.7 Glass Formation

The glass transition has a dramatic effect on frozen food quality. The product is most stable below maximal-freeze-concentration temperature (i.e. T_m', T_g''', or T_g'), and moisture has little influence on these temperatures. The presence of low molecular weight solutes lowers the T_g', and high molecular weight solutes exert little effect. This means that with increasing maturity many vegetables display a decrease in sugars and an increase in starch, thus increasing the maximal-freeze-concentration temperature and the stability of frozen foods.

39.4.8 Functional Properties

The functional properties of any food product are normally affected by differences in freezing and thawing methods. Properties such as rheological, both flow and dynamic, texture, mechanical, consistency, appearance and sensory attributes, and water-holding capacity, among others, have been related to freezing and thawing processes. The changes in specific functional properties become microscopically related to structural modifications or rearrangements of the food items. The rheological properties of fresh and frozen–thawed okra dispersions were significantly different when measured

at 20–80°C. The dispersions were pseudoplastic with both the consistency coefficient and flow behavior index influenced by temperature. The consistency coefficient was higher for unfrozen sample than frozen–thawed sample when measured at 20 and 50°C, but the reverse was observed at 80°C. The flow behavior indices were not different at any temperature between 20 and 80°C [55].

Navarro et al. [56] studied the effect of the freezing rate on the rheological response of gelatinized starch pastes containing lipids. Low freezing rates increased the viscoelasticity, reduced the apparent viscosity, and led to higher structural changes of the pastes; in contrast, high freezing rates maintained the same rheological characteristics of the unfrozen samples. Graiver et al. [57] evaluated the viscoelastic behavior of refrigerated and frozen mozzarella cheese, finding differences in the viscoelastic response. The storage modulus was higher for refrigerated samples in comparison with frozen cheese, in which modifications in cheese microstructure by freezing were observed with SEM methodology. Further, the freezing was related to casein hydrolysis, cheese matrix softening, and proteolysis acceleration.

Texture is important in frozen vegetables [58, 59]. After freezing-thawing, firmness decreased, rupture strain increased, and consequently crispness decreased [58, 60]. The rate of freezing was critical to tissue damage. The optimum freezing rate of carrots was established as –5°C/min using a programmable freezer and based on texture and histological structure [61, 62]. Chinese cabbage leaves crack when frozen rapidly with liquid nitrogen. The optimum freezing rate for Chinese cabbage was 2°C/min considering tissue softening and drip loss. Freezing and thawing accelerated the release of pectin, but the freezing rate did not greatly affect pectin release [58]. Fruits, such as strawberries, apples, peaches, and citrus, contain thin-walled cells with a large proportion of intracellular water, which can freeze, resulting in extensive cell rupture and radical alteration of the mechanical properties of the material [63]. Maestrelli et al. [59] studied the effect of freezing on three quality parameters of transgenic parthenocarpic (parthenocarpy produces seedless fruits) eggplants, in which the firmness of the transgenic eggplants showed a decrease. As the tissue of apple and potato freezes, ice crystals form extra- or intracellularly, pushing the cells apart or rupturing cell walls and producing large voids within the tissue. Changes in mechanical behavior (wedge penetration, tensile, and compression) of the material were directly related to the degree of cell damage by freezing [64].

Mashl et al. [65] studied the unidirectional freezing of a gelatinized corn starch–water mixture and found that at freezing velocities less than or equal to 7.5 μm/s, starch granules were alternately pushed or entrapped by the advancing solid–liquid interface. This produced a segregated structure consisting of alternating high-starch and low-starch bands; thus segregation of the starch within the product occurred, which is detrimental to consistency, texture, and appearance. At a velocity of 10 μm/s, the frozen product was homogeneous.

The development of rancid flavors and progressive toughening accompanied by the development of cold store flavor are the principal sensory changes in seafood [18]. Deterioration in the texture and functionality of fish tissues by frozen storage takes place faster than in other animal muscles [47]. The firming of the soft texture characteristic of young fish with the early onset of protein denaturation is preferred by most taste panelists [66]. Flavor change is probably more critical than texture since this can occur early [67]. The denaturation of myosin increased in a frozen solution. The rate of formation of insoluble, high molecular weight protein aggregates increased as the temperature decreased below the freezing point and reached a maximum near the eutectic point of the solution [68]. Because of the concentration effect, pH can also change during freezing. A decrease in pH to more favorable values for degradation results in faster protein denaturation [7]. Fish gradually loses its juiciness and succulence after freezing and subsequent frozen storage. In gadoids, the chemical breakdown of trimethylamine oxide (TMAO) to dimethylamine (DMA), and formaldehyde (FA) and the subsequent cross-linking of FA to muscle proteins produced textural breakdown and resulted in a cottony or spongy texture. In this case, free water exists loosely like a sponge. When eaten, the fish muscle loses all its moisture during the first bite, and subsequent chewing results in a very dry and cottony texture [46]. In non-gadoid species, such as crab, shrimp, and lobster, muscle fibers also tend to toughen and to become dry during freezing and storage.

Thawing and refreezing could lead to quality deterioration [4]. Dyer et al. [69] reported accelerated deterioration for refrozen cod fillets stored at –23°C. Changes in enzyme activities of α-glucosidase and β-*N*-acetylglucosaminidase in rainbow trout on thawing, refreezing, and frozen storage were observed, but they did not relate to differences in sensory attributes [70]. Cowie and Little [71] reported no correlation between decreasing protein solubility and sensory attributes for cod stored at –29°C. Thawed and refrozen fish muscle displayed a faster decline in myofibril protein solubility than once-frozen fish and had reduced water-holding capacity, but analysis of proton spin-spin relaxation times indicated no changes in water location. The decline in protein solubility was not caused by complete protein unfolding. Long thawing times of 30 h before refreezing and storage resulted in cooked fish having a gray appearance and stale flavor [4]. Whole fish when thawed exhibits less textural change than filleted fish due to the presence of the backbone, which provides structural support for the flesh [46]. Gaping in fish fillet may be observed to worsen if fish are slowly frozen. Love and Haq [72] showed that the rate at which whole cod were frozen had little effect on the gaping of the fillets cut after thawing, although very slow freezing did cause a small increase [72].

The functional properties of cheese, which also changed after freezing and thawing, include meltability, stretchability, elasticity, and free oil formation, cohesiveness, and others with a specific meaning. Meltability is the capacity to flow together and form a uniform continuous melted mass. Stretchability is the tendency upon pulling to form fibrous strands that elongate without breaking. Elasticity is the capacity of the fibrous strands to resist permanent deformation. Free oil formation

is the separation of liquid fat from the melted body into oil pockets. Viscosity is due to particle segregation or coagulation. Luck [73] concluded that frozen storage was suitable for cream cheese, unripened camembert, and brick cheese, but not for Gouda or cheddar cheese. Mozzarella cheese, originated in Italy, is consumed worldwide largely due to the popularity of pizza and similar foods. Mozzarella differs from most cheeses in that it is often consumed in a melted state. Consequently, the functional properties, such as meltability, stretchability, elasticity, and free oil formation, are important to the quality of the product. Cervantes et al. [74] concluded that freezing (one-week storage) and thawing did not affect the mozzarella cheese quality as assessed by compression, beam bending, and sensory evaluation. Dahlstrom [75] showed poor meltdown, acid flavor, fat leakage, free surface moisture, bleached discoloration, and poor cohesiveness immediately after thawing, but normal characteristics reappeared after the thawed cheese was aged for 1 to 3 weeks. Bertola et al. [76] studied the freezing rate and frozen storage (3 months at –20°C) of mozzarella cheese. The functional quality loss (meltability, apparent viscosity, free oil formation) can be avoided as long as the product is aged from 14 to 21 days at 4°C before being consumed. The frozen mozzarella cheeses that had been ripened for about 14 days produced a product similar (hardness, adhesiveness, cohesiveness, springiness, and non-protein nitrogen) to refrigerated cheese at the same stage of aging [40].

39.5 CHEMICAL CHANGES AND QUALITY

39.5.1 Rancidity

Oxygen is the bugbear of almost all frozen foods, leading to oxidative rancidity (if any unsaturated lipids are present), loss of color, and development of off-flavors. Freezing results in a concentration of solutes, which catalyze the initiation of oxidative reactions, and disrupt and dehydrate cell membranes, exposing membrane phospholipids to oxidation. Membrane phospholipids are highly unsaturated and have been demonstrated to be the initiation point of oxidation in muscle tissue [2].

The degradation of lipids in frozen peas during storage at –18°C led to flavor damage due to the formation of hydroperoxides, thiobarbituric acid, and fatty acids, particularly in unblanched samples [77]. The lipid oxidation was mainly due to lipoxygenase and lipohydroperoxidase breakdown products [78]. The hydrolyzed and oxidized products of lipids affect the quality of frozen vegetables [79].

Oxygen availability and tissue composition play important roles in the acceleration of lipid oxidation of frozen fish. Lipid hydrolysis occurs in fish during storage, which may affect lipid oxidation. Free fatty acids are more readily oxidized than the equivalent esters when lipoxygenase is involved [80–82]. Lipid oxidation in mackerel minces occurred continually as long as the samples were exposed to air independent of hydrolytic activity, but was deactivated or retarded by cooking the sample or by lowering the storage temperature (–40°C). Lipid oxidation was observed not only in the free fatty acids but also in the triacylglycerides and the phospholipids extracted from mackerel mince [83]. Mincing destabilizes the fish due to a high level of incorporated oxygen and cellular disruption, making the lipids susceptible to oxidation. Most methods for frozen fish mince stabilization are based upon one or several of the following three strategies: (i) removal of pro-oxidants, oxygen, or components susceptible to oxidation, (ii) alternation of pro-oxidants, antioxidants, or other components influencing the oxidation, or (iii) addition of components that can protect fish mince lipids against oxidation [84].

Washing of fish mince helps the removal of various antioxidative components and the relative increase in both polarity and un-saturation in remaining lipid fraction. Heating also affects the oxidative stability of fish mince by (i) altering pro-oxidative enzymes, such as lipooxygenases [85], lipooxidases [86], and microsomal enzymes [87], (ii) changing the pro-oxidative properties of myoglobin and other hemoproteins [88], and (iii) enhancing the production of aqueous [85] and lipid-soluble [89] antioxidants. Undeland et al. [90] studied the lipid oxidation stability of frozen minced herring at –18°C by pre-heating and pre-washing. Stability increased due to heat inactivation of catalytic enzymes without activation of hemoproteins. Washing reduced the stability by removing pro-oxidative enzymes from cooking and caused a reduction of antioxidants as well as a relative increase in phospholipids and free fatty acids in the fat. In the case of herring fillets, the removal of the skin prior to storage at –18°C was shown to affect the stability of underlying tissue negatively. The abundance of hemoproteins, free metals, and enzymes in the under-skin layer resulted in very severe oxidative changes, especially when the fillets were stored skinned. The unfavorable composition of the under-skin layer includes a lot of dark muscle, the silver surface, and the highest fat content and the lowest level of α-tocopherol. Such local productions of oxidation products can spoil the entire flavor of the fillet; protection or removal of unstable tissues at an early stage during processing can be important factors in the improvement of frozen herring storage stability [90].

Poultry fat becomes rancid during very long storage periods or at extremely high frozen storage temperatures. Rancidity in frozen whole poultry stored for 12 months is not a serious problem if the bird is packaged in essentially impermeable film and held at –18°C or below. The danger of rancidity is greatly increased when poultry is cut up before freezing and storage because of the increased surface exposure to atmospheric oxygen [44]. Antioxidants, like BHA, BHT, or tocopherols, and metal chelators such as pyrophosphates, tripolyphosphates, or hexametaphosphates are effective in reducing oxidation. The distribution of antioxidants in meat is difficult; thus including tocopherols in animal feed results in the deposition of tocopherols in membrane locations. This is much more effective in preventing the initial step with phospholipids [2]. The frozen storage stability of antioxidant-treated beef heart surimi is reported [83, 91]. Among various lipid- and water-soluble antioxidants, propyl gallate was found most protective and effective for inhibiting lipid and protein oxidation during short-term frozen storage [92]. Lipid

and protein oxidation in beef surimi with propyl gallate and cryoprotectants (sucrose, sorbitol) was minimal at –70°C. The freezing-thawing process caused lipid and protein oxidation in the frozen sample at –15 and –29°C. Oxidation increased rapidly after 4 weeks. Propyl gallate inhibited lipid oxidation but was ineffective against protein changes. After 12 weeks cryoprotectants promoted lipid and protein oxidation in the absence of propyl gallate [93].

Malonaldehyde is one of the decomposition products of auto-oxidation of polyunsaturated lipid materials in food. Malonaldehyde is the main component in the 2-thiobarbituric acid (TBA) value that is used to evaluate the degree of oxidation of lipids. Malonaldehyde reacts with myosin from trout. The rate of reaction of malonaldehyde with α-amino groups of myosin was greater at –20°C than at 0°C and almost equal to that of 20°C [8].

The oxidant level should be increased in frozen dough formulations, as oxidants increase dough strength. A higher shortening level is recommended for frozen dough production. Generally, shortening protects the dough structure from damage due to ice crystallization [41].

39.5.2 Color, Flavor, and Vitamin Loss

39.5.2.1 Color Loss

The most important color changes in fruits and vegetables are related to three biochemical or physicochemical mechanisms [94]: (i) changes in the natural pigments of vegetable tissues (chlorophylls, anthocyanins, and carotenoids), (ii) development of enzymatic browning, and (iii) breakdown of cellular chloroplasts and chromoplasts. Pineapple for processing should be of optimum ripeness with a yellow color, good aroma and flavor, and free from blemishes, such as black heart, water blister, yeasty rot, or brown spot. For frozen pineapple slices, semi-translucent, highly colored slices are generally considered the most attractive and have the best flavor. Pineapple color is important because it is often the basis for judging product acceptability. The golden color of pineapple fruit is mainly due to carotenoids, which become more predominant with ripening as chlorophyll content decreases. Heat processing, freezing, and thawing lead to cell disintegration, pigment degradation, and isomerization of carotenoids [95–97].

Color and flavor are important sensory attributes, and vitamin content is a functional attribute of frozen foods. The green color of vegetables is lost by the chlorophyll degradation during freezing and frozen storage resulting from the conversion of chlorophyll to pheophytins and/or to the destruction of both chlorophyll and pheophytins, giving a dull khaki color. During storage, chlorophyll is converted to pheophytin with a loss of green color and vitamin C; these can be used as objective indicators of quality [18, 29, 98].

Chlorophyll was bleached during fat peroxidation, oxidation of glycolic acid and by α-hydroxy acid dehydrogenase, and chlorophyllase, which hydrolyze the phytal ester group of chlorophylls and phephytins [99]. Storage temperature and time, acidity, and blanching time affect the loss of chlorophyll in frozen vegetables. A ten-fold increase in the conversion rate occurred with an approximate 8.3°C increase in temperature. Blanching decreased the loss of chlorophyll during frozen storage [29]. Various inorganic salts, such as sodium chloride, potassium sulfate, sodium sulfate, and sodium or ammonium bicarbonate, have been used to reduce chlorophyll loss [94].

The maximum stability of carotenes in frozen spinach was 2 years at –28.9, 1 year at –6.7, and 7 days at 4.4°C [100]. Carotene retention curves were sigmoidal with three regions: Initiation, acceleration, and retardation. They were typical of autocatalytic reactions. Lipoxygenases were the major enzymes involved in carotene degradation [101]. Moharram and Rofael [29] reviewed carotene degradation in frozen vegetables. Martins and Silva (2004) reported a high sensibility of chlorophylls (color a and b values) at –18°C, and results showed that color a and b values retained only 10.96 and 10.82% for chlorophylls during 60 days of frozen storage.

In poultry, a light surface color for carcasses is considered important and is best achieved with rapid surface freezing, which generates a smooth chalky white surface. This is achieved by supercooling the product and forcing nucleation of a high number of small ice crystals. These crystals stay small because there is little water migration to already formed crystals during such a fast process. Numerous small ice crystals cause the surface to reflect light and appear white in color [2]. An alternative approach is to crust freeze the outer part of the carcass rapidly using liquid brine immersion, spray systems, or cryogenics like liquid nitrogen or carbon dioxide, and then to move the partially frozen bird to air blast or cold storage for the remainder of the process. A freezing front migration rate of 2–5 cm/h is recommended to achieve fast freezing effects and 0.1–0.2 cm/h for slow freezing [2, 52].

Darkening of bones is a condition that occurs in immature chickens and has become more prevalent as broilers are marketed at younger ages. Darkening may arise during chilled storage or during the freezing and defrosting process. It occurs because some of the heme pigment normally contained in the interior of the bones of particularly young chickens leaches out through spongy areas and discolors the adjacent muscle tissues [2, 44]. Leaching only occurs in carcasses from relatively young birds because the bones are not completely calcified and are more porous than in mature birds [2]. The development of dark bones was greatly reduced by a combination of freezing and storage at –35°C and immediate cooking after rapid thawing [102]. Aside from this combination, the freezing rate, time between slaughter and freezing, temperature and length of storage, and temperature fluctuations during storage have no marked influence in preventing this discoloration [44]. While eating qualities do not change, the appearance constitutes a negative factor in consumer acceptance [2].

In crustaceans, a dark discoloration defined as blackspot or melanosis is developed after the trauma of capture, freezing, and thawing process, and it is unattractive to consumers, reducing the market value. This oxidative enzyme reaction, followed by auto-oxidation and polymerization, may be prevented by the application of sulfiting agents in combination with freezing [103]. Rotland et al. [103]) carried out an

experiment in which different concentrations of sodium metasulfite (included in HQ-Bacterol F), temperature and time of immersion, and subsequent freezing storage of rose shrimp were applied. They found that untreated shrimps showed melanosis after 19 hours of ice and with a decreased market value at 27 h, whereas samples treated with 2% HQ-Bacterol F for 5 min maintained their market value. Further, quick freezing appeared to be a good method in addition to the sulfiting agent to prevent the melanosis phenomenon, and following storage for 3 months did not affect the appearance of blackspot.

39.5.2.2 Flavor and Aroma Loss

Freezing affects the flavor and aroma of frozen foods. For example, the freezing of strawberries is usually associated with a reduction in aroma and the development of off-flavor. The decrease in aroma is due to the rapid decomposition and diffusion of esters [104, 105], whereas the concentrations of franeol and mesifurane linked to strawberry flavor are not affected by freezing [105]. The off-flavor of frozen strawberries differs from that of frozen vegetables [106–108]. Off-flavor in frozen vegetables is usually due to insufficient blanching. Deng et al. [109] found that the development of off-flavor in frozen–thawed strawberries was due to the chemical production of H_2S rather than enzymatic activity. The identity of H_2S was verified both chemically and using gas chromatography–mass spectroscopy analyses. The olfactory properties detected by sensory analysis indicated the presence of sulfurous compounds. Usually, H_2S is derived from the sulfur-containing amino acids cysteine or methionine during processing. Deng et al. [109] also showed that amino acid was not the main precursor of the off-flavor compounds, but the off-flavor development in frozen strawberries can be attributed to the breakdown of the cells by freezing, thereby decreasing the pH in the cytosol, which in turn leads to the release of sulfide ion as H_2S. The duration of the production of H_2S was longer in strawberries at –40 and –80°C than at –20°C. This may be due to the low boiling point of H_2S (–59.0°C). Vigorous crushing of fresh strawberries also gave rise to the production of H_2S. Thus, structural damage is one of the important factors.

In fish and seafood, formaldehyde is formed during cold storage by the enzymic decomposition of TMAO. It is a good objective criterion of time–temperature exposure in frozen gadoid species [110]. The formaldehyde reacts with proteins, thereby decreasing their solubility in salt and buffer solutions [18, 92]. Santos-Yap [46] mentioned that changes in the flavor of fish and seafood generally occurred in three distinct phases during frozen storage: (i) gradual loss of flavor due to loss of or decrease in concentration of some flavor compounds, (ii) detection of a neutral, bland, or flat flavor, and (iii) development of off-flavors due to the presence of compounds such as acids and carbonyl compounds that are products of lipid oxidation.

39.5.2.3 Vitamin Loss

The retention of nutritional components in foods is a concern when any type of preservation method is used, but freezing is probably the least destructive [2]. The destruction of vitamin C (ascorbic acid) occurs during freezing and frozen storage. This loss is influenced by blanching conditions, types of freezing, package, and time–temperature conditions [29]. The loss is mainly due to the oxidation and/or to the action of ascorbic acid oxidase [79]. A ten-fold increase in the rate of loss of ascorbic acid per 8.9C rise in storage temperature of frozen vegetables was found [111]. Generally, frozen vegetables stored at –24°C displayed better ascorbic acid retention than those at –12 and –18°C, respectively [29]. Blanching improves ascorbic acid retention in vegetables. A combination of microwave energy and steam or water blanching yielded frozen products with better ascorbic acid retention than conventional procedures [112]. The reduction in vitamin C in frozen mashed potatoes could be overcome by the addition of encapsulated vitamin [113]. Vitamin B losses sometimes occur in frozen meat products. B vitamin losses may be significant in frozen poultry products, but most losses are the result of the subsequent thawing and cooking treatments rather than of the freezing process [2]. Martins and Silva [98] suggested that the shelf-life determination of frozen vegetables should depend on nutritional quality rather than only sensory attributes.

39.5.3 Release of Enzymes

The disruption of plant or animal tissues by freezing leads to the release of enzymes bound to the structures. Beef and pig skeletal muscles contain two isozymes of glutamic-oxalacetic transaminase: One in the mitochondria and the other in the sarcoplasm [114]. Hamm and Kormendy [115] found that freezing and thawing causes a remarkable increase of glutamic-oxalacetic transaminase activity in the muscle press juice. Fish contains malic enzymes in two forms: Free and latent. The latter is solubilized by the disruption of the tissue caused by freezing and thawing [116]. Barbagli and Crescenzi [117] found that the activity of cytochrome oxidase in extracts of tissues after freezing and thawing was increased by 15 times in chicken liver, by 2.5 times in trout, and by four times in beef muscle compared to extracts of unfrozen samples. Thus, a method was developed to distinguish between fresh and frozen meat based on the enzymes released [115, 117–119]. Around 0°C enzymic breakdown of protein becomes the principal cause of quality loss, and below –8°C microbiological spoilage ceases and protein denaturation coupled with oxidative rancidity in fatty species become the chief factors affecting quality [18].

39.5.4 Hydrolysis

Generally, starch in vegetables does not change significantly during frozen storage [120]. Rofael [79] observed no significant changes in starch of beans, peas, okra, or mallow during storage at –18°C for 1 year. The reducing sugars of these frozen vegetables were increased during storage due to the hydrolysis of both oligo- and polysaccharides of these products. Thus, the amount of reducing sugars is a good indicator of storage life [29]. Martins and Silva [98] found significant

starch degradation rates for green bean at –6, –12, and –18°C during the initial days of storage. In melons, total cell wall polysaccharides decreased more during the first 5 months than during the second 5 months of frozen storage. This suggested that pectin and hemicellulose fractions were modified and solubilized by either mechanical or enzyme-catalyzed changes in cell wall polymers [121]. However, freezing preservation of pineapple slices led to minimal changes in soluble solids and sugar content (fructose, glucose, and sucrose), pH, titratable acidity, and nonvolatile organic acids (citric and malic acids) after a year of frozen storage at –18°C [122, 123].

39.5.5 Acetaldehyde Formation

The formation of acetaldehyde in frozen vegetables increases during storage; thus it is an indication of shelf life [79]. Acetaldehyde is a product of aerobic fermentation of pyruvate in plant tissues [124]. The amount of acetaldehyde formation depends on the pretreatment, such as blanching time and storage period [29, 79, 125]. Chow and Watts [125] found that acetaldehyde increased when fresh vegetables were heated beyond the minimum required for enzyme inactivation. Dimethylamine content, formaldehyde content, and shear force measurement correlated very well with the sensory texture score of frozen red hake [126]. The enzymatic breakdown of TMAO to dimethylamine (DMA) and formaldehyde affects textural changes in fish species during frozen storage. Further, formaldehyde's contribution to protein changes in muscle during frozen storage would clarify the toughening mechanism. Frozen storage and fluctuation in temperature affect both dimethylamine and formaldehyde formation in frozen fish [127].

39.6 COMPARISON BETWEEN DIFFERENT FREEZING METHODS

Bartolome et al. [128] evaluated the influence of freezing (cold room –18°C and air-blast freezer –50°C) and frozen storage (–18°C for 0 to 12 months) on the color and sensory quality of pineapple slices (Smooth Cayenne and Red Spanish cultivars). No differences were found in sensory analysis (color and appearance) between the cultivars, frozen at different rates, compared to fresh product, or after 1year of frozen storage. However, both cultivars were suitable for freezing. The freezing methods (liquid nitrogen, –120°C and stored at –40°C; individual quick freezing at –43°C and stored at –40°C; and conventional deep freezing and storage at –20°C) showed total color changes as 15.5, 29.0, and 38.2% respectively in frozen date fruit stored for 9 months [129]. Similarly, instrumental texture profile analysis (TPA) parameters decreased during storage; for example, after storage for 9 months hardness decreased to 21.0 N from the initial 119.5 N in the case of liquid nitrogen followed by 30.0 N for quick freezing and 42.2 N for conventional freezing. Similarly, polyphenol activity increased from 0.034 to 0.147, 0.184, and 0.287, respectively while peroxidase increased from 0.039 to 0.054, 0.063, and 0.163, respectively [129]. However, liquid nitrogen and quick freezing showed no significant difference. Sucrose decreased from 21.8 g/100 g sample to 12.3, 5.1, and 0.0, respectively, while glucose increased from 5.0 g/100 g sample to 7.9, 10.7, and 13.0, respectively, and fructose increased from 4.6 g/100 g sample to 7.2, 7.6, and 7.7 after 9 months of frozen storage [129].

39.7 PRETREATMENTS FOR FREEZING

It is important to realize that successful freezing will only retain the inherent quality present initially in a food item and will not improve quality characteristics; thus the quality level prior to freezing is a major consideration. The use of high-quality initial materials based on standards and grades is essential to high-quality frozen products. The levels of intrinsic product quality measures, such as freshness, suitability of variety or genetics for freezing, soil nutrients for foods of plant origin, and dietary factors for foods of animal origin, are affected. In addition, harvesting or slaughtering methods, and processing such as blanching, cooking, chilling, and the addition of antioxidants also have profound effects. Microbiological quality prior to freezing remains a major determinant to post-thaw quality. Although freezing can reduce some pathogens, there is also usually significant survival. Thus, other methods must be used to ensure the elimination of pathogenic organisms from frozen poultry [2, 130]. The commonly used pretreatments are discussed in the following sections.

39.7.1 Blanching

Most vegetables and some fruits are blanched before freezing. Blanching destroys the semi-permeability of cell membranes, destroys cell turgor, removes intercellular air, filling these spaces with water, and establishes a continuous liquid phase. As a result, ice crystallization can occur through the entire matrix of food without interruption during the freezing process. Blanching also affects texture, color, flavor, and nutritional quality by inactivating enzymes. Cell turgor is an important component of the eating quality of many fruits. It is produced by the internal pressure of cell contents. Reduced turgor is perceived as softness and lack of crispness and juiciness. When turgor is an important product characteristic, blanching and freezing may not be acceptable. If the product is cooked before consumption, the retention of turgor through earlier processing is not necessary since thermal treatment is more severe than blanching or freezing [6, 29]. Blanching also has other advantages, such as the destruction of microorganisms, and wilting of leafy vegetables, assisting packaging [3, 94]. Further, blanching favors reductions of some undesired compounds that are present in some leafy vegetables, such as nitrates, nitrites, and oxalates [131]. The effect of blanching, at 70°C for 15 or 30 s, and frozen storage on the stability of -carotene and capsanthin in red pepper was elucidated by Morais et al. [3]. Both time of blanching and frozen storage were simultaneously included in two multilinear equations describing the concentration of -carotene and capsanthin. There were significant differences in the decomposition rate of pigment related to cultivars and process conditions.

Properly blanched vegetables have a long shelf life at frozen food temperatures enabling them to be exported all over the world and to span the seasons [18]. Blanching of fruits may be detrimental in many cases, resulting in (i) rapid discoloration by enzymatic browning [18], (ii) loss of texture, (iii) formation of cooked taste, (iv) some loss of soluble solids, especially in water blanching, and (v) adverse environmental impact due to energy requirements and disposal of used water [94]. Blanching at 70–105°C associated with the destruction of enzyme activity. Hot water blanching is usually carried out between 75 and 95°C for 1 to 10 minutes, depending on the size of the individual vegetable pieces. High-pressure steam blanching is more energy-efficient than water blanching. It is important that cooling be carried out shortly after blanching, especially for products to be frozen [94].

The enzymes involved in the production off-flavor are catalase, lipoxygenase, and peroxidase, and their heat stability varies with the types of vegetables and fruits. Peroxidase and catalase seem to be the more heat stable, and thus could be used as an index of adequate blanching for vegetables. A 95% loss of enzyme activity following blanching is considered adequate. The quality of blanched frozen vegetables was improved if some peroxidase activity remained at the end of the blanching process. The activities of most enzymes are greatly dependent on the pH of the tissue or the blanching water. Additives, such as citric acid, sodium chloride, and carbonates, can be used in water depending on the purpose [29, 94, 132]. Bottcher [133] reported that the highest-quality products were obtained when the following percentages of peroxidase activity remained: Peas, 2–6.3%; green beans, 0.7–3.2%; cauliflower, 2.9–8.2%; and Brussels sprouts, 7.5–11.5%. It was concluded that the complete absence of peroxidase activity indicated overblanching [94].

39.7.2 Heat Treatments

Texture is an important quality attribute of frozen fruits and vegetables. Loss of tissue firmness, disruption of the cell membrane, and excessive softness are the major consequences to be avoided [134]. Low temperature and long-time pretreatment were useful in improving the texture of frozen vegetables by avoiding excessive softness. Carrots heated for 30 min at 60°C and frozen above –5°C/min (optimum rate) should escape both cell damage and excessive softening [62, 135]. The de-esterification of pectin by pectin-methylesterase during preheating prevented transelimination of pectin [136–139]. Fuchigami et al. [140] found that preheating followed by quick freezing was effective in improving excessive softness and cell damage. The optimum preheating occurred with 30 minutes at 60°C or 5 minutes at 70°C, and the optimum freezing was at –5 to –50°C. Preheated carrots retained a firmer texture than those blanched in boiling water. After preheating, the amount of high-methoxyl pectin decreased and low-methoxyl pectin increased. The quick freezing process resulted in better texture than slow freezing.

Loss of texture was accompanied by the release of pectin. Slow freezing accelerated the release of pectin as compared to quick freezing. Preheated carrots were slower to release pectin. The degree of esterification of pectin substances in raw carrots decreased during preheating, freezing, and thawing. Cell damage in quick-frozen carrots was slight. Product preparation prior to freezing may include cutting, deboning, slicing, and other operations to provide greater convenience. A greater variety of products cooked prior to freezing are becoming popular with consumers. These include breaded and fried portions, cured and smoked products, and items in marinades or broths [2]. The freezing rate of precooked chicken affects the quality of the product. Breaded precooked drumsticks frozen with liquid nitrogen are susceptible to cracking, separation of meat from the bone, and development of small areas of white freezer burns next to the surface [44]. Cooked products are likely to exhibit greater increases in lipid oxidation than raw products during storage. This is due to the oxidative change and higher TBA values making the product more susceptible to further oxidative changes during frozen storage. Antioxidants are very effective in stabilizing cooked chicken during frozen storage [141–143].

39.7.3 Dipping Pretreatments

In many cases, foods are dipped or soaked in different solutions before freezing, and the type of solute used depends on the desired purpose. Apple slices are commonly treated by soaking in 1% salt solution in order to remove intercellular air. Fruits are also dipped in ascorbic acid and sugar solutions to minimize browning or blanched for a short time to inactivate enzymes [6]. Paredi et al. [50] studied the effect of dipping in polyphosphates on the biochemical properties of adductor muscles during freezing and storage at –30°C. Immersion in polyphosphates solution was effective in reducing water loss in stored muscle. In addition, it delayed the decrease in enzymatic activity (Mg^{2+}–ATPase) and provided some protections for the myosin light chains without affecting either the extractability or the viscosity of actomyosin from frozen stored muscles.

In many cases, the frozen product is protected by a suitable glazing compound. A glaze acts as a protective coating against the two main causes of deterioration during storage: dehydration and oxidation. It protects against dehydration by preventing moisture from leaving the product, and against oxidation by mechanically preventing air contact with the product. Oxidation can also be minimized if the glaze carries a suitable antioxidant [44]. For products intended for short-term storage, glazing can be practically utilized as a viable alternative to storage without a protective covering [46]. Moreover, glazing treatment could be a cheaper alternative to expensive packaging systems for fish stored at –20°C [144]. The different glazes available include inorganic salt solutions of sodium acid phosphate, sodium carbonate, and calcium lactate, alginate solution, also known as *Protan* glaze, antioxidants, such as ascorbic and citric acids, glutamic acid, and monosodium glutamate, and other edible coatings, such as corn syrup solids [145].

39.7.4 Bacterial Ice Nucleators or Antifreeze Proteins

The application of bacterial ice nucleators to the freezing of some model food systems and real foods, such as salmon, egg white protein, and cornstarch gels, elevates nucleation temperatures, reduces freezing times, and improves the quality, such as flavor and texture. These can also be used in freeze concentration of fresh foods for the modification of their properties [146]. The use of bacterial ice nucleators is a unique application of biotechnology, as it directly improves freezing processes [147]. When bacterial cells were added to isotropic aqueous dispersions of hydrogels composed of proteins and polysaccharides, the bulk of the water was converted into directional ice crystals at subzero temperatures not lower than –5°C, and resulted in the formation of anisotropically textured products [147]. Details of this topic are reviewed by Wolber [148] and Li and Lee [147]. Antifreeze proteins, found in polar fish and cold-tolerant insects and plants, can affect freezing in several different ways: (i) by lowering the freezing temperature, (ii) by retarding recrystallization on frozen storage, and (iii) by promoting ice nucleation causing supercooled solutions to freeze more rapidly [12, 149]. Mizuno et al. [42] studied the effect of solutes on the antifreeze and immobilizing activities of water. The antifreeze activities of saccharides that consisted of glucose were higher than others, and in salts those that possessed a higher ionic charge had higher antifreeze activities. In water-soluble amino acids, a few amino acids that formed no eutectic mixture above –20°C had especially high antifreeze activities. The high antifreeze activity is caused by high immobilizing activity for water molecules, and the immobilizing mechanism varied with the type of solute [42]. The antifreeze proteins depress the freezing point by attaching to ice crystals and interfering with water molecules joining the ice lattice. Computer modeling suggests that at least for one antifreeze peptide, the molecules are arranged in an antiparallel fashion with cooperative side-to-side-binding [150].

There are two groups of antifreeze proteins: Antifreeze glycoproteins and antifreeze proteins. The primary structure of the antifreeze glycoproteins is a repeating (Ala–Ala–Thr) sequence with a disaccharide attached to the threonine residue. The antifreeze proteins have various structures. Type I proteins have an α-helical structure, whereas type II and III proteins have some unusual secondary structures. Synthetic anti-freeze peptides may have also practical applications in foods [150]. The phenomenon of cold acclimation in carrots was related to biochemical and physiological changes in the fresh plant [149]. The presence of antifreeze protein within the roots was related to cold acclimation and its quality enhancement through freezing.

39.7.5 Osmotic Concentration

Partial removal of water by osmotic treatment to freezing is recognized as convenient for reducing cellular damage of fruits and vegetables, which causes softness after thawing [151]. Osmotic concentration of vegetables prior to freezing is a pretreatment that can improve end-product quality [94, 151]. It is well-established that osmotic dehydration improves the product quality in terms of color, flavor, and texture. The merits of osmotic dehydration for product-quality improvement and process efficiency have been reviewed earlier [152–154]. The effects of sugar on the quality of frozen fruits have been reviewed by Skrede [155]. However, in the literature there is not much fundamental information about the mechanisms of flavor entrapment in the food matrix, color retention, and physics of textural improvement. In the frozen food industry, high energy levels are used for freezing because a large quantity of water is present in fresh foods. A significant proportion of this energy could be saved if plant materials were concentrated prior to freezing [156]. A reduction in the moisture content of food can reduce refrigeration load during freezing. Partially concentrating fruit and vegetables prior to freezing saves packaging and distribution costs [157]. The product quality is comparable with that of conventional products. The process is referred to as dehydro-freezing.

39.7.6 Cryoprotection

Meat and fish muscle is susceptible to freeze denaturation, which decreases gel-forming potential, water-holding capacity, and protein solubility. Cryoprotectants are generally added to protect fish myofibrillar proteins from freeze denaturation during frozen storage [158]. Polydextrose, sucrose, and sorbitol have been reported to protect against freeze denaturation of Alaskan pollack surimi [159]. These are low in cost, safe, and have good solubility and beneficial functional effects [160]. Sucrose is usually combined with sorbitol to reduce sweetness. The cryoprotective effect of sugar is enhanced by adding polyphosphate [161]. Polydextrose proved to be an effective cryoprotectant for both pre- and post-rigor beef. Arakawa and Timasheff [162] found that cryoprotectants increase the surface tension of water as well as the binding energy, preventing withdrawal of water molecules from the protein and thus stabilizing the protein. Phosphates had no cryoprotective effect but did increase pH and enhanced protein extractability, which may enhance gel-forming and water-holding properties [52].

Park et al. [158] found that cooked gel strength was unaffected by the freezing of beef or pork surimi-like materials for 48 h, and the addition of cryoprotectants (3 or 6% sorbitol, 3% glycerol, or 3% sucrose) before freezing had no effect on gel-forming ability. The washed myofibrillar proteins from beef muscle were quite stable during freeze-thaw treatment up to 6% sodium chloride. No difference in gel-forming ability after freezing with or without added salt was found. Wierbicki et al. [163] also reported no detrimental effects on water-holding capacity due to salting of meat prior to freezing. The interaction between salt ions and muscle proteins occurs rapidly, compared to the normal process of shrinking or coagulation of muscle proteins [164]. Dondero et al. [165] studied the cryoprotective effects of 18, 20, 25, and 36 DE maltodextrins at 8% (w/w) in surimi from jack mackerel stored at –18°C for

27 weeks. They found that 20 and 25 DE maltodextrins as well as sucrose or sorbitol mixtures were the most effective in stabilizing surimi proteins during frozen storage [165].

Poultry meat showed little deterioration upon freezing, and isolated myofibrillar systems made by the surimi procedure are less stable [160]. Kijowski and Richardson [166] found that mechanically recovered meat from broilers had reduced functionality when no cryoprotectants were used. Sorbitol or sucrose showed some protection of the gel-forming ability of frozen samples, and sorbitol or sucrose with tripolyphosphate gave stronger gels after freezing or freeze-drying than fresh samples. The combined presence of sorbitol, sucrose, and tripolyphosphate restored most functional properties of frozen or freeze-dried material to the levels of the fresh material. Most of the loss of functionality during freezing or freeze-drying was caused by loss of solubility of myosin and, to a lesser extent, actin. Freeze-drying had a greater effect when no additive or NaCl was present. The blast-frozen and freeze-dried samples with no cryoprotectants had a very coarse structure with no obvious fine network system. In the presence of sorbitol or sucrose there was a finer meshwork for freeze-dried material, which was finer for frozen material. In the presence of sorbitol or sucrose with tripolyphosphate, the network was even finer but with fewer obvious spaces in the matrix for both freeze-dried and frozen material. These were observed by scanning electron microscope.

Whole egg and yolk products are fortified with salt or sugar before freezing to prevent coagulation during thawing. The selection of additive depends upon the finished product specifications. Salt (10%) is added to yolks used in mayonnaise and salad dressings, and sugar (10%) is added to yolks used in baking, ice cream, and confectionery. Egg whites are not fortified as they do not have gelation problems during defrosting [44]. Table 39.1 shows the effects of freezing on the functional properties of liquid egg products [167]. High molecular weight (HMW) polymer cryoprotectants have the following advantages over low molecular weight (LMW) cryoprotectants [168]:

1. HMW polymers do not generally penetrate the cell membrane and remain in the extra-cellular suspensions and/or outer surface of the cell.
2. HMW polymers do not produce a significant freezing point depression within the range of concentrations that can be applied in practice.
3. There is no binary eutectic, i.e. the hydrated polymer does not crystallize from aqueous solution as LMW agents do.
4. HMW polymers have the ability to keep a substantial portion of the solution from freezing.

Although the presence of HMW polymeric cryoprotectants is limited to the extracellular suspension medium, HMW additives affect the intracellular composition by the efflux of intracellular water due to chemical potential change across the membrane when extracellular ice is formed [168].

39.7.7 Irradiation

High-dose irradiation can produce changes in the chemical composition and taste of fish and seafood. A combination of irradiation and freezing can be used in foods. A combination treatment involving freezing in conjunction with irradiation has recently been proposed as a means of retarding spoilage. It has been reported that parts of Europe irradiated frozen seafoods from Asia to eliminate microbial pathogens such as *Salmonella*.

39.8 STORAGE AND DISPLAY

Packaging, storage, and display also affect frozen food quality. Loss of quality in frozen foods is a gradual process,

TABLE 39.1
General Effects of Freezing Rate, Storage Time, Storage Temperature, Thawing Rate, and Additives on Functional Properties of Liquid Egg Products

	Effect on Functional Properties		
Factor	**Egg Albumen[a]**	**Egg Yolk[b]**	**Whole Egg[c]**
Freezing rate	Slower rate causes: reduced viscosity and increased foam stability	Slow rate causes: increased viscosity and gelation	Same as liquid egg yolk but less severe
Storage time	Longer time causes: reduced viscosity and increased foam stability	Longer time causes: increased viscosity and gelation	Same as liquid egg yolk but less severe
Storage temperature	Lower temperature causes: reduced viscosity and less severe	–18°C results in maximum increase in viscosity and increased foam stability	Same as liquid egg yolk but gelation
Thawing rate	Faster rate causes: some protein denaturation less severe	Slower rate causes: increased viscosity and gelation	Same as liquid egg yolk but gelation
Additives	None normally needed	2% NaCl and 8% sucrose	None normally needed inhibits gelation; 10% used commercially

Source: Dawson [167].

[a] Freezing usually has only a slight effect on egg albumen properties.

[b] Freezing often has a drastic effect on egg yolk viscosity.

[c] Freezing has a greater effect on whole egg properties than albumen but less than the effect on egg yolk.

the changes being slow or very slow, cumulative, and irreversible [18]. Optimum quality requires care in every stage of the processing, packaging, storage, and marketing sequence. The storage temperature is important for frozen food. Symons [18] mentioned that the speed of freezing was not as important to product quality as the maintenance of adequately cold temperatures (–18°C or less) during distribution. A package for frozen products should (i) be attractive and appeal to the consumer, (ii) protect the product from external contamination during transport and handling, and from permeable gases and moisture vapor transfer, (iii) allow rapid, efficient freezing and ease of handling, and (iv) be cost-effective. To provide the greatest protection, a package must be well-evacuated of air (oxygen) using a vacuum or gas-flushed system and provide an adequate barrier to both oxygen and moisture [2, 44]. Since cost is involved in vacuum or modified-atmosphere packaging, these should only be used when necessary for quality. For example, vacuum packaging need not be used if lipid oxidation is not the limiting factor affecting the shelf life of a product.

The shelf life of frozen foods kept in open display cabinets at –15°C packed in 23 different types of plastic, cardboard, and laminate was studied. It was found that aluminum foil-laminated and metallized packages gave the best results. This is due to low levels of oxygen permeability, water vapor transmission, and light transparency, and less fluctuating temperatures [169]. Two terms used to describe the shelf life of frozen foods are practical storage life (PSL), and just noticeable difference (JND). Practical storage life is the level of quality expected for the product by the ultimate consumer. Just noticeable difference is usually determined by a trained taste panel and then multiplied by an arbitrary figure, generally between 2 and 5, to arrive at a practical storage life [18]. In some sensitive products, such as peaches, cauliflower, and red-pigmented fish, the PSL may be close to the JND [18]. Most frozen products enjoy a shelf life of many months or even years [18].

Quality losses of frozen food increase log-linearly with the storage temperature when greater than –18°C [170]. The rate of quality loss increases about 2–2.5 times for every 5°C increase over –18°C [5, 51, 171]. In poultry, it has been suggested that shelf life is likely to change by a factor of 3.5 for each 10°C change, up or down [2]. In seafood kept at around 0°C, enzymic breakdown of protein becomes the main cause of quality loss; below –8°C microbiological spoilage ceases, and protein denaturation coupled with oxidative rancidity in fatty species become the chief factors affecting quality [18]. Some types of foods, such as fish, pork, animal organs, fried chicken, and spinach, can be maintained in a high-quality state for only 3–7 months at –20°C, whereas other foods, such as beef, sugared fruits, many bakery products, and many vegetables can be maintained in a high-quality state for more than 12 months at –20°C [10]. Fish stored at –29°C will have a shelf life of more than a year [44]. The practical storage life values are determined by the International Institute of Refrigeration (IIR) by Symons [18].

39.9 THAWING

Thawing as a final and obligatory step of the freezing process is quite important. Thawing properly is essential to maximize quality retention and safety of frozen foods. Microbiologically safe thawing processes are (i) inside a refrigerator at temperatures below 5°C, (ii) microwave oven, or (iii) as part of the cooking treatment [98]. Although thermal processing in microwave and cooking assures better microbial destruction when compared with thawing inside a refrigerator, sensory quality retention is compromised. In a study on green beans quality loss upon thawing, Martins and Silva [98] found that sensory parameters, such as flavor and color, were more sensitive to thawing at refrigeration temperature (3–7°C), than nutritional properties, such as vitamin C and starch contents. In a study of Virtanen et al. [172], the thawing time of a model food system, based in wheat flour, was reduced to seven parts, when they combined microwave energy and cold air in comparison to convective thawing at ambient temperature, but no quality changes were quantified. High pressure, microwave, ohmic, and acoustic thawing are innovative applications that are being explored to improve conventional thawing methods. High-pressure methods preserve food quality and reduce the necessary time for thawing, but some inconvenient characteristics have been mentioned, such as high costs, protein denaturation, and meat discoloration [173]. Similarly, microwave, ohmic, and acoustic thawing may require shorter thawing times, but some limitations have been found. Heterogeneous heating, controlled frequencies, and many more investigations need to be considered with these new thawing methods [173].

39.10 COLD CHAIN TOLERANCE AND QUALITY

39.10.1 Temperature Cycling

The steps in the frozen food cold chain are freezing, transport by refrigerated vehicle or container, distribution store, retail display cabinet, the unrefrigerated period between retail outlet and home, and time in a home freezer before being consumed in the frozen state, thawed, or end cooked. Temperature abuse at any of the above steps causes quality deterioration. Time-temperature indicators have been proposed to monitor the lack of adequately cold temperatures during the cold chain. Fluctuations in storage temperature may contribute to deterioration of frozen foods [29].

39.10.2 Time–Temperature Tolerance Indicators

The concept of time–temperature tolerance (TTT) to describe frozen food stability is important. Physicochemical, chemical, or biological reactions give an irreversible indication (usually visible) of the history of the product. These indicators are placed on the outside of the packages and combine the time and temperature conditions to which they have been exposed [94]. Temperature history indicators do not provide a precise record of temperature as it changes with time, as do time-temperature recorders or digital data acquisition systems, but

are less costly [174]. Indicators that respond continuously for all temperature conditions are said to be full-history indicators, whereas devices that respond only for the period of time during which a temperature threshold has been exceeded are called partial-history indicators [174]. A more detailed review of time–temperature indicators is given by Taoukis et al. [175]. The applicability and effectiveness of time–temperature integrators (TTI) as monitors and controlling tools for frozen chain and distribution of frozen vegetables, green peas, and mushrooms were assessed by Giannakourou and Taoukis [176]. In this analysis, TTI response provided a reliable indication of the relative quality status of the frozen products, in which these TTI tools may be utilized as a base to optimize the management system and consequently to improve consumer acceptance [176].

39.11 EMERGING FREEZING TECHNOLOGY

Freezing process can be used as one of the hurdles in combined methods of food preservation. Piotrowski et al. [177] carried out a study with osmotic dehydration, freezing, and microwave convective drying to preserve strawberries. In the case of apple cubes, an osmotic dehydration treatment followed by freezing provided good quality and acceptance. The optimum conditions were found to be 55°Brix, 35°C for the solution, and 60 min of osmotic dehydration time with fast freezing [178]. Sun and Li [13] and Li and Sun [179] applied ultrasound with freezing to potato tissues, which resulted in an improved freezing rate. Higher freezing rates produced better cellular structure, less intercellular void formation, and less cell disruption when studied by cryo-scanning microscopy analysis. The application of power ultrasound was effective in improving the structure of frozen-then-thawed potatoes. A combination of dehydration in concentrated solutions and freezing was applied to muskmelons [180] and strawberry slices [151] in order to identify improvement in texture and structure. Moisture reduction of muskmelon, prior to the freezing process, improved the quality by reductions in exudate loss and texture after thawing [180]. Also after thawing, the pre-dehydrated strawberry samples exhibited a better tissue organization than the frozen slices without pretreatment, the best texture corresponding to the air-dried and the osmotic concentrated-air dried strawberry samples [151]. Fagan et al. [181] utilized a modified atmosphere packaging, with different N_2/CO_2 ratios, combined with freeze-chilling, to extend the shelf life of raw whiting, mackerel, and salmon, finding that these combined technologies confer logistical benefits, not only during frozen storage, also in product distribution and retailing.

Recently, Zhan et al. [182] reviewed the emerging freezing technologies to improve the quality and safety of frozen foods. These include high-pressure freezing, electrically and magnetically assisted freezing, ultrasound-assisted freezing, and utilization of antifreeze protein. These have been developed to minimize the disadvantages of traditional freezing methods, which cause damage to the cell structure, increased drip loss, and poor sensory value. At high pressure and different temperatures water forms different states of ice, and high pressure can be classified into three types: (i) high-pressure induced freezing, (ii) high-pressure shift freezing, and (iii) high-pressure assisted freezing [183, 184].

An electric field enhances the orientation or rotational movements of polar molecules, and thus it affects water molecules under electric field [185]. It causes the early formation of ice crystals due to the minimization of free energy, and enhances the nucleation process [186, 187]. Therefore, electric field or electric disturbance during freezing showed positive effects on the quality of frozen foods [188, 189]). The magnetism of water molecules forms closer molecules as a higher number of hydrogen bonds are formed. This makes the network more stable with increased thermal conductivity and a raised freezing point of water, thus controlling supercooling with delayed ice formation [190–192]. Similarly, microwave-assisted and radiofrequency-assisted freezing are being tested for their potential use in foods [182]. These can induce the dipole rotation of water molecules and cause disturbance in ice nucleation, formation, and growth. This is due to the rearrangement of the hydrogen bonds with an applied electric field, which makes a stronger water network [193, 194]. The ultrasound-assisted freezing has also been tested for foods. The cavitation and micro-streaming effects generated by ultrasound intensify heat transfer, promoting the formation of ice crystal and fracturing large ice crystals [179, 195]. Zhan et al. [182] mentioned in his review that all these methods have more or less positive effects on meat, especially controlling ice crystals by creating smaller, homogeneous, and regular-size crystals as compared to the conventional ones. They ultimately better preserve microstructure and quality. However, more confirmatory works need to be done to explore both positive and negative dimensions of these new technologies.

39.12 CONCLUSION

Freezing is one of the commonly used methods of food preservation, and it provides relatively better quality as compared to drying and canning. The varied rate of freezing causes the formation of different types of ice crystals, thus affecting the structure of the frozen and thawed foods. The detrimental effects of freezing on the microorganisms depend on the types of pathogens, spoilage, and beneficial microbes. Freezing changes free and bound waters in foods, moisture loss, recrystallization, retrogradation, protein denaturation, freezer burn, glass formation, functional properties, hydrolysis, acetaldehyde formation, oxidation, color, flavor, aroma, and vitamins. Different pretreatments, such as blanching, heat treatment, dipping treatments, and osmotic drying, can improve the quality of frozen foods. In addition, the utilization of bacterial ice nucleators, cryoprotectants, and antifreeze proteins is emerging. The emerging technologies, such as electric and magnetic field, radiofrequency, microwave, ultrasound and high pressure, are being explored for controlled ice formation and for enhancing the quality of frozen foods.

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40 Freezing Methods of Foods

Jorge Fernando Velez-Ruiz and Mohammad Shafiur Rahman

CONTENTS

40.1 INTRODUCTION

Food preservation by freezing is a process based on the effect of low temperatures in a range of –2 to –18°C for commercial purposes, although lower temperatures can be used. It was conceived to preserve perishable foods for long periods of storage with good quality. However, there are significant differences in the shelf life of frozen foods when the storage is carried out at –12°C (or higher temperatures) compared to at –18°C. Freezing as natural phenomenon is very old and as a transformation process is one of the conventional food preservation technologies. The invention of a freezing unit by Reece in 1867 used ammonia as the refrigerant. In 1893, a load of 60,000 tons of salmon were transported from Kamchatka to United States. Clarence Birdseye, a food researcher and trader, developed freezing plate equipment, and in 1931 this pioneer founded a company to market frozen foods.

There are two main factors involved in food preservation by freezing; one is the increasing of osmotic pressure or water activity reduction, due to concentration of solutes, and the second is maintaining the low temperature through the storage. The concentration of solutes is achieved through crystallization of water, which contributes to prevent microbial growth and to destroy microbial cells to some extent. In addition, there are fewer deteriorative reactions due to the high viscosity of the concentrated solution. Maintaining the low temperature during storage is very important, because both biochemical and chemical reactions as well as microbial growth are minimized, and it contributes to the high quality of frozen products [1].

It is very interesting to observe that the economic growth of developing countries is quantified on the basis of how many people have a domestic refrigeration unit. On the other side, people from developed countries may freeze almost all foods, because they have at least one freezing unit. Raw food materials change with time, the overall cost for freezing preservation being lower than that for canning and/or drying if the freezer can be kept full [2–4]. Different pretreatments, such as blanching, sulfating, and cooling, are used to reduce the freezing time as well as to produce high-quality frozen foods. The design or selection of freezing equipment implies the knowledge of several aspects and fundamentals, such as the freezing point, heat requirements, processing times, and relation between unfrozen water as a function of temperature. This chapter presents different types of freezing methods with their descriptions, advantages, and limitations.

40.2 FUNDAMENTALS OF THE FREEZING PROCESS

The freezing process may be divided into three stages, based on the temperature evolution; in the first stage, or "cooling" (above the freezing point), the temperature of the food is decreased from its original condition or initial temperature to the freezing point (Figure 40.1). In the second stage, or properly freezing (at constant temperature), as a natural phenomenon the freezable water changes from liquid to solid state. Finally, the third stage or "subfreezing" (below the freezing point): food decreases its temperature from its freezing point to the storage temperature, and the ice formation increases. These three stages can be observed in Figure 40.2, which shows the freezing curve for three different foods (i.e. three levels of water content); the first curve was developed for a vegetable with high water content (97.8%) and consequently exhibited a freezing point (–0.10°C) close to water. The second

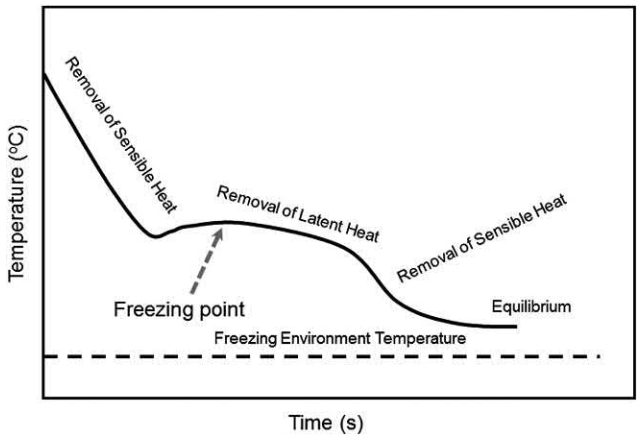

FIGURE 40.1 Typical temperature–time curve for a freezing phenomenon.

curve corresponds to a dispersion (mixture of milk and solids from nut) with medium water content (64.9%); then its freezing point is also close to that of water but lower (–1.24°C). Whereas the third, a mixture of several ingredients in a paste, had a low water content (13.5%) and showed a very low freezing point (–35.2°C) [3]. Therefore, the best type of freezing method for the specific food item needs to take into account its freezing point as well as complete engineering design. It is important to estimate the refrigeration load of a freezing process based on the fundamentals of heat transfer. Figure 40.3 shows the heat transfer and movement of the frozen layer during the freezing process. We can mention three important aspects that affect the calculation (Figure 40.1); the first one is related to the decrease of temperature (sensible heat) and phase change (latent heat) of the food (Q_F), and the other two, maintenance (Q_M) and losses (Q_L), implying sensible, latent, convective, and conductive heat transfer phenomena. The chamber maintenance requires a heat loss estimation during the storage of frozen foods. The freezing rate depends on the convective heat transfer coefficient and thermal conductivity. A typical range of surface heat transfer coefficient is 5–2000 W/m^2 K [4–6] and thermal conductivity ranges from 0.5 to 1.5 W/m K [4, 7]. Even though the focus of this chapter is not engineering, some process parameters (h, the convective heat transfer coefficient and the latent heat of fusion) and physical properties (k, conductivity, and C_p, specific heat) related to the freezing of foods are included in Table 40.1.

40.3 SLOW AND QUICK FREEZING

A specific freezing method is characterized by two physical phenomena: an initial nucleation or ice generation, followed by crystal growth of the ice cores. The quality of the frozen foods mainly depends on the size of the ice crystals generated through the whole process; thus the rate of freezing is recognized as critical to achieving quality in frozen foods. Therefore, the different methods applied in the food industry may be part of a slow-freezing group or a quick-freezing group. A rapid freezing process generates small intracellular

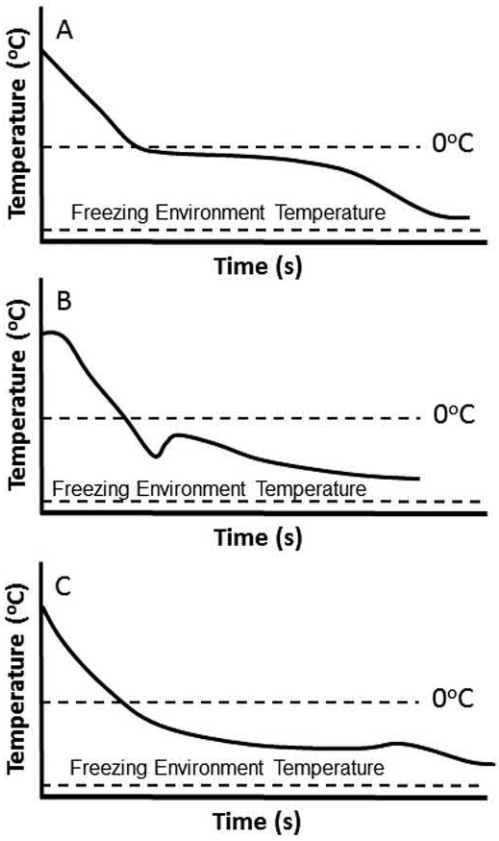

FIGURE 40.2 Temperature–time curve for the freezing of three foods, A: high water content (a vegetable), B: medium water content (a dispersion), C: low water content (a paste).

ice crystals; in contrast slow freezing produces large ice crystals (Figure 40.4). There are several process characteristics that are considered to distinguish both groups; these are emphasized in Table 40.2.

There are different types of freezing systems available for foods. No single freezing system can satisfy all freezing needs because of the wide variety of food products and process characteristics. The selection criteria of a freezing method are the type of product, reliable and economic operation, easy cleaning ability and hygienic design, and desired product quality. Although most of the commercial freezing processes are operated at atmospheric conditions, there are potential applications of high-pressure assisted freezing and thawing of foods, or the use of vacuum conditions [8–11]. The pressure-induced freezing point and melting point depression enables the sample to be super-cooled to low temperatures (e.g. –22°C at 207.5 MPa), resulting in rapid and uniform nucleation and growth of ice crystals on release of pressure. Other results include increased thawing rates, the possibility of non-frozen storage at subzero temperatures, and various high-density polymorphic forms of ice. Details of the applications of this process are reviewed by Kalichevsky et al. [12].

The International Institute of Refrigeration (IIR) used four categories for food freezing based on speed, defined as the "cold front." This is visualized as the line or front that separates the frozen part from the unfrozen part of the food item

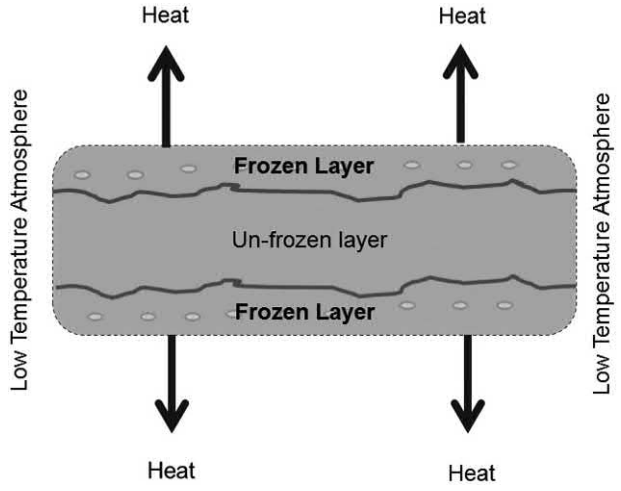

FIGURE 40.3 Heat transfer and frozen layer during the freezing process.

(Figure 40.3). Slow freezing with a front speed of 0.1–0.2 cm/h is used for bulk freezing inside big rooms, quick freezing with a speed of 0.5–3.0 cm/h for blast and plate freezers, rapid freezing with a range of 5–10 cm/h in fluidized units, and ultra-rapid freezing with a front speed of 10–100 cm/h is reached in cryogenic and liquid spray freezers [10, 11].

In the food industry, plate contact, immersion, air blast, fluidized-bed, and cryogenic freezing are common methods, and although these have particular performance characteristics, the number and size of crystals formed during freezing involve several factors, such as the quantity of product, specific properties of the food, characteristics of the particular method, operation conditions of the equipment, heat transfer, and presence of packaging, among others [4, 11].

The quality of the frozen foods depends of the following factors: (i) quality of the fresh food, (ii) application of pretreatments, (iii) selection of the best freezing method, (iv) hygienic process conditions, (v) right freezing speed, (vi) presence of packaging, and (vii) proper thawing. Thawing is very important and transcendent because an incorrect thawing process causes poor quality in frozen foods.

40.4 CONVENTIONAL FREEZING METHODS

Freezing methods may be grouped in four types: (i) freezing by contact with a cooled solid surface (plate freezing), (ii) freezing by contact with a cooled liquid (immersion freezing), (iii) freezing by contact with a cooled gas (e.g. cabinet freezing), and (iv) freezing by cryogenic medium.

TABLE 40.1
Process Parameters and Physical Properties of Some Freezing Systems and Food Products

Freezer Type	Conditions	h (W/m² K)	Example Foods Preserved by the Method	
Cold room	Still air	5–10	Beef carcass, chicken, fruits, vegetables	
Air-blast	Air velocity: 2.5–5 m/s	15–30	Fruits, vegetables, fish fillets	
Tunnel	Counterflow of food item and air	15–60	Grains, soybean, fish fillets	
Fluidized-bed	Suspending airstream	80–120	Carrot cubes, peas, shrimp, strawberries	
Plates	Contact to solid	50–120	Meat steaks, fish fillets, leafy vegetables	
Cryogenic	Gas zone/spray zone	40–60/100–140	Ice cream, shrimp, berries	
Liquid immersion	Circulating brine	60–90	Chicken, turkey, canned foods	
	Specialized refrigerant	500–1200	Fruits, tomato slices, orange segments	
Food item	k (W/m K)	Cp above and	below freezing (kJ/kg K)	Latent heat of fusion (kJ/kg)
Apples	0.513 (before freezing, water 84.9%)	3.65	1.90	281
Bananas	0.481 (before freezing, water 75.7%)	3.35	1.78	251
Chicken		3.32	1.77	247
Ice cream	0.460 (before freezing, at 0°C)	2.95	1.63	210
Milk (whole)	0.473 (before freezing, water 87.0%)	3.79	1.95	294
Oranges	0.580 (before freezing, water 85.9%)	3.75	1.94	291
Shrimp	0.490 (before freezing, water 75.3%, fat 1.2%)	3.62	1.89	277
Strawberries	0.462 (before freezing/1.125 (at –15.5°C)	3.86	1.97	301
Tomato (ripe)	0.571 (before freezing, water 92.3%)	3.99	2.02	314
Turkey	0.343 (before freezing, water 92.8%, fat 12.4%)	2.98	1.65	214
	1.437 (water 92.8%, fat 12.4%, at –9.4°C)			
	1.627 (water 92.8%, fat 12.4%, at –23.3°C)			
Watermelon	0.571 (before freezing, water 92.8%)	3.96	2.01	311
Water	0.594 (before freezing, at 0°C)	4.23 (at 0°C)	2.01	334

Sources: Desrosier and Desrosier [40], Singh and Heldman [17], Cengel and Boles [41], Velez-Ruiz and Soriano-Morales [42], Velez-Ruiz [4].
h: heat transfer coefficient (W/m² K), k: thermal conductivity (W/m K), C_p: specific heat (kJ/kg)

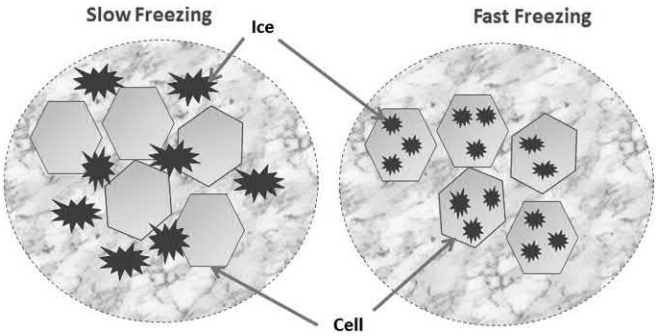

FIGURE 40.4 Location and size of ice formation during slow and fast freezing processes.

TABLE 40.2
Main Characteristics for Slow and Quick Freezing Methods

Process Characteristic	Slow Freezing	Quick Freezing
Temperature decreasing	≤ 2°C/min	≥ 10°C/min
Crystal size	Higher size	Lower size
Removed (lost) water	Higher lost	Lower lost
Dripping water	Higher dripping	Lower dripping
Protein denaturation	Higher denaturation	Lower denaturation
Cell tissue damage	Higher destruction	Lower destruction
Structure integrity	Lower integrity	Higher integrity
Water holding capacity (WHC)	Lower WHC	Higher WHC

Sources: Modified from Heldman and Singh [29], and from Velez-Ruiz [4].

40.4.1 Freezing by Contact with Solid Surface (Plate Freezing)

In this method, the product is placed between metal surfaces or plates, cooled either by refrigerants, cold brines, or even other fluids (Figure 40.5). The plate adjustment by hydraulic pressure is usually utilized for good contact with the food surface. The thermal conductivity of metals used for the contact plates is high, being important from the heat transfer viewpoint; however, the manufacture of these plates is importantly governed by sanitary requirements. Plate freezers are only suitable for regular-shaped food materials or blocks, such as fish or meat, as they favor good contact between the surfaces of the plates and the food item.

When the product has been frozen, hot liquid is circulated to break the ice seal and defrost. Spacers should be used between the plates during freezing to prevent crushing or bulging of the package. In the freezing of liquid foods, such as vegetable purees, fruit juices, food concentrates, and ice cream, cylindrical scraped-surface or vertical freezers may be used. When the freezing process is completed, the plates are separated and the frozen food product is removed, for reloading of the next batch into the unit equipment. This equipment may be operated in batch, semi-continuous, or continuous arrangements. The

FIGURE 40.5 Photo of plate freezing equipment (manufacturer: Jackstone).

filled freezer looks like a multilayered sandwich with alternative layers of cold plates and food product.

The number of parallel plates may be separated by up to 9 cm as a function of their position, in a number of 5–20 with a contact area of 1.2–2.5 m^2, for instance plates with dimensions of 0.8×1.5 m^2 or 1.1×2.0 m^2. These freezers may have a capacity of 6000 to 15,000 kg/d or specific capacity of 160 kg/m^2 h. A cabinet with 20 plates has overall dimensions of $2 \times 2 \times 3$ m^3, with weight of 1.8–2.0 tons. One of the important advantages of this type of freezing is its capacity, being two to four times higher than that of the tunnel units [11].

There is a particular type of indirect-contact freezer known as a scraped-surface heat exchanger. It is used mainly for viscous liquid foods, such as ice cream. In this system, a cylindrical tube and a rotor are in the inside part of the equipment, whereas in the external part there exists another tube or a jacket, surrounding the tube surface with refrigerant flowing inside. Around 60–80% of the latent heat is removed from the food, and it leaves the equipment as a frozen slurry [4, 13].

40.4.2 Freezing by Contact with Cooled Liquid (Immersion Freezing)

In this method, food is immersed in a low-temperature brine and nontoxic mixtures of solutes and water (aqueous solutions). Direct contact with liquid could achieve fast temperature reduction through direct heat exchange [9]. The fluids usually used are salt solutions (calcium chloride and sodium chloride), sugar solutions, and alcohol solutions (glycol and glycerol). The solutes used must be safe for the product in terms of health, taste, color, and flavor, and the product must be denser than the fluids. Dilution from the foods may change the solution properties; thus it is necessary to control the concentration.

Due to the direct contact between the cooling medium as a liquid and the food item, the heat transfer is very effective. In order to ensure that the food does not come into contact with some liquid refrigerants, flexible membranes or packaging can be used to enclose the food completely while allowing rapid heat transfer [14]. The water loss and salt gain, respectively, were less than 2 and 1 g/100 g for gelatin gel in immersion

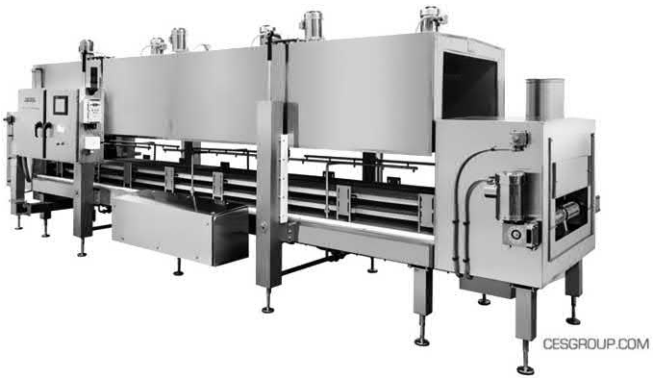

FIGURE 40.6 Air tunnel freezing with a conveyer belt for holding and carrying food (manufacturer: CESGROUP).

freezing with sodium chloride solution, and the salt penetration was hindered by the formation of an ice barrier [15]. A mixture of glycerol and glycol is a liquid–liquid medium that can be used since there is no eutectic point for the solutes. As the temperature is lowered, a point is reached where ice crystals are formed as slush. The temperature at which slush ceases to flow is called the flow point. Methanol or ethanol can also be used. Although the methanol will be removed during cooking, it is poisonous, whereas ethanol is safe. Alcohols also pose a fire hazard in processing plants.

40.4.3 Freezing by Contact with a Cooled Gas (e.g. Cabinet Freezing)

In this method, air in a chamber is utilized. These systems attempt to put the air into contact with as much of the food surface as possible. As the cooling medium by natural convection with a lower surface heat transfer coefficient or blown as forced convection with higher convective, and forced convection air may be used in tunnels (Figure 40.6), a conveyor belt (Figure 40.7), and fluidized-bed equipment. The convection of large quantities of air, due to its low specific heat, may be blown horizontally or vertically to the flow of the food products. For static air in which heat transfer is lower than for forced air, the convective heat transfer coefficient is a function of the air velocity. High velocities are more effective for the freezing of unpackaged food items, particularly when the foods are completely surrounded by the flowing gas.

40.4.3.1 Room or Cabinet Freezing

In this method or system, cold air is circulated inside the room or cabinet, depending of the food quantity, where the food product is placed in trays (e.g. fish fillets) or suspended (e.g. half beef carcasses). Room and cabinet freezers have been used for almost all types of food. Fresh or packaged foods can be frozen in air at temperatures of –18 to –40°C. The natural convection phenomenon of air plays an important role in the freezing speed (i.e. high velocity determines higher heat transfer). In these systems, moisture pick-up from the product surface may deposit on the cooling coils as frost, and acts as an insulation. A cabinet freezer with air velocity of at least 5 m/s generates high heat-transfer rates [9]. When the air is forced or blown through a particular equipment geometry or design, the method is known as blast freezing.

Soyer et al. [16] carried out a study to observe the effect of freezing temperature (–7, –12, and –18°C) and storage time (up to six months at –18°C) on the oxidation of lipids and proteins in chicken meat. They found not significant the effect of the freezing temperature, but found storage time to have an important effect on both oxidation phenomena after three months, and they detected a higher reaction intensity in leg meat than in breast meat.

40.4.3.2 Air-Blast Freezing

In this method, the temperature of food is reduced by cold air flowing at a relatively high velocity. The application of this system is limited to some foods, in which the residence time is low to avoid and control moisture loss through the process. The use of packaging for foods helps to avoid this problem. Air velocities between 2.5 and 5 m/s give the most economical freezing. Lower air velocities result in slow product freezing, although higher velocities increase unit freezing costs considerably [8]. It is useful for foods with a variety of shapes and sizes. This method can use a simple design, and the existing

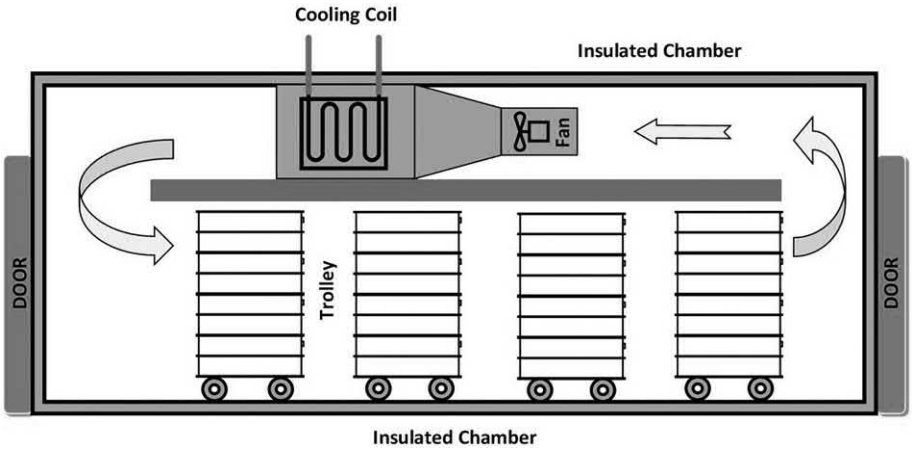

FIGURE 40.7 Air tunnel freezing with trolleys or pellets for holding and carrying food.

designs are divided into tunnel freezing, belt freezing, and fluidized-bed freezing, depending on how air interacts with the product [9, 17].

Silvas-Garcia et al. [18] followed the effect of freezing rate on the solubility, structure, and rheology of gluten proteins and bread quality. The frozen dough at a slow freezing rate exhibited a decrease in the proportion of insoluble polymeric protein and gas retention, as well as resistance to extension, whereas an increase in protein solubility and viscous modulus was measured, in contrast to the dough frozen at rapid rates. In addition, an increase in hardness and a decrease in springiness and specific volume were observed with fast freezing. These changes in properties are very important because the quality of bakery items is related to the viscoelastic properties of the gluten proteins. Santos-Goncalvez et al. [19] also compared the effect of freezing with static and forced air on bioactive compounds and antioxidant activity of strawberry pulp. They found that forced air was better for retaining phenolic compounds and the antioxidant activity of the pulp, where the levels of polyphenol oxidase and peroxidase enzymes were stable. Additionally, as a good advantage for the static air, the freezing method did not affect the anthocyanin levels.

40.4.3.2.1 Tunnel Freezing

In this process, the products are placed in trays or racks in a long tunnel where the cool air is circulated over the product (Figure 40.7). There are single-, double-, and multiple-row tunnels. Due to equipment performance, it may be used for any food item, from fine-cut or minced foods up to whole turkeys and even half beef carcasses. Three basic elements of the tunnel freezer are the push-through device, the trays, and the chain drive system with very good insulation. The tunnel has an entrance door and an exit door for continuous operation, with air temperature of –30 to –40°C and velocity of 3–10 m/s, and it requires powerful fans and large heat exchangers. The freezing time depends on the process conditions in addition to the size and thermal conductivity of the food product; it usually lasts 1.5–6 h, with a capacity of around 85 kg/m^2 h, and obviously, lower times imply higher costs. The advantages of this method are its versatility for food handling, its simplicity and easy cleaning, but it has important disadvantages, such as higher moisture loss (~3%), and the need for larger space and labor in comparison with other freezing methods. Mendez [20] carried out a study to find the effect of freezing and storage on the weight loss of meat. A weight loss of 0.05–1.20% was found depending on the animal part (i.e. sides and quarter of beef and pork), when tunnel freezing with air velocities of 0.8–3 m/s and temperatures of –20 to –30°C is used.

40.4.3.2.2 Belt Freezing

The first mechanized air-blast freezer consisted of a wire mesh belt conveyor in a blast room for continuous product flow (Figure 40.6). It consists of long belts moving through cold airstreams in a parallel or countercurrent arrangement. Uniform product distribution over the entire belt is required for the product, favoring a high surface contact and effective freezing. The systems include a horizontal straight belt, and up-and-down belt, and a curved belt. Controlled vertical airflow forces cold air up through the food product layer, thereby creating good contact with the item and increasing the thermal efficiency. The principal current design is a two-stage belt freezer. Temperatures are usually –10 to –4°C in the precool section and –32 to –40°C in the freezing section [8].

40.4.3.2.3 Spiral Freezing

A spiral belt freezer is a variant of the belt and tunnel systems; it consists of a long belt wrapped cylindrically in two tiers, thus requiring minimal floor space (Figure 40.8). The spiral freezer uses a conveyor belt that can be bent laterally. It is suitable for products with a "long" freezing time (generally 10 min to 3 h), and for products that require gentle handling during freezing. It also requires a spatial air-distribution system [8].

40.4.3.2.4 Fluidized Bed Freezing

A fluidized-bed freezer consists of a bed with a perforated bottom through which refrigerated air is blown vertically upwards in a compacted cabinet. The air velocity must be greater than the fluidization velocity (i.e. value at which the food is suspended/maintained in the air stream). This freezing method is suitable for small pieces or particulate foods of fairly uniform size (2.5–12.5 cm) by forming a bed of peas, diced carrots, potatoes, corns, and berry fruits, and giving sense to individual quick freezing (IQF), where the food units are frozen individually. An air temperature of –34°C is common. The high degree of fluidization improves the heat-transfer rate and results in short freezing times and good use of floor space. Sheen and Whitney [21] found that the surface or convective coefficient was two to three times higher in a fluidized bed than with convective air in a freezer for large food items. This method has several advantages, such as short freezing times, high heat transfer, less food dehydration, and less need for equipment defrosting. It has the limitation that large or non-uniform food units cannot be handled in this type of freezing equipment. Similar characteristics may be assumed for cryogenic units.

FIGURE 40.8 Commercial spiral freezer (Nantong Baoxue Refrigeration Equipment Co.).

40.4.4 Freezing by Immersion and Cryogenic Materials

Food items have direct contact with the freezing medium, similar to air systems, in which the freezing process is completed very quickly, generating frozen food products of superior quality. A liquid refrigerant is utilized, undergoing phase change as the freezing occurs. The food product to be frozen is either immersed in the liquid or sprayed with it, therefore the foods might be protected with an adequate package.

40.4.4.1 Immersion Cryogenic Freezing

In this method, a solution is used (immersion methods) by the application of liquid carbon dioxide (CO_2), nitrogen (N), or Freon fluids (cryogenic methods), with boiling points of –79, –196, and –30°C (Freon 12), respectively. The product is immersed in cryogenic liquid in an immersion freezer.

40.4.4.2 Cryogenic Freezing

In cryogenic freezing, liquefied gases are used in direct contact with the foods, either packaged or unpackaged. Food is exposed to an atmosphere below –60°C through direct contact with liquid nitrogen or liquid carbon dioxide. Other compounds, such as CCl_2F_2 (Freon 12) and N_2O, are also used. Due to their thermodynamic properties and easy handling, nitrogen is mainly used in food processing plants. It is usually used for high-value products due to the high capital cost for gas compression. Very high heat transfer (i.e. highest h) may be achieved with this freezing method, due to the easy and quick evaporation of the cryogenic liquid.

The product can be exposed to a cryogenic medium in two ways: (i) the cryogenic liquid is directly sprayed on the product in a tunnel freezer (Figures 40.9 and 40.10), and (ii) the cryogenic liquid is vaporized and blown over the food in a spiral freezer or batch freezer [1, 4, 11, 22, 23].

Freezing with nitrogen is a very fast method (2.5 times faster than fluidized-bed freezing, for example); thus adequate control is necessary for achieving quality products. It also provides flexibility by being compatible with various types of food products. The rapid formation of small ice crystals greatly reduces the damage caused by cell rupture, preserving color, texture, flavor, and nutritional value. The rapid freezing also reduces dehydration of the products, provides high product throughput, and has low floor space requirements. Thermal diffusivity of the food, however, restricts the heat transfer from the product to the freezing medium [9, 11, 14]. Spraying of the cooling medium is conveniently applied in continuous units, and it is more widely used than direct immersion in the cryogenic fluid.

Cryogenic gases can also be advantageously applied to produce a hard, frozen crust on a soft product to allow for easier handling, packaging, or further processing [24]. The cryo-mechanical technique utilizes a cryogenic gas to create a frozen crust on a fluid product, after which the product may then be conveyed to a conventional mechanical freezer. The combination of these processes offers the advantages of both systems [14]. The advantages of liquid nitrogen are: it is colorless and odorless and is chemically inert and boils at

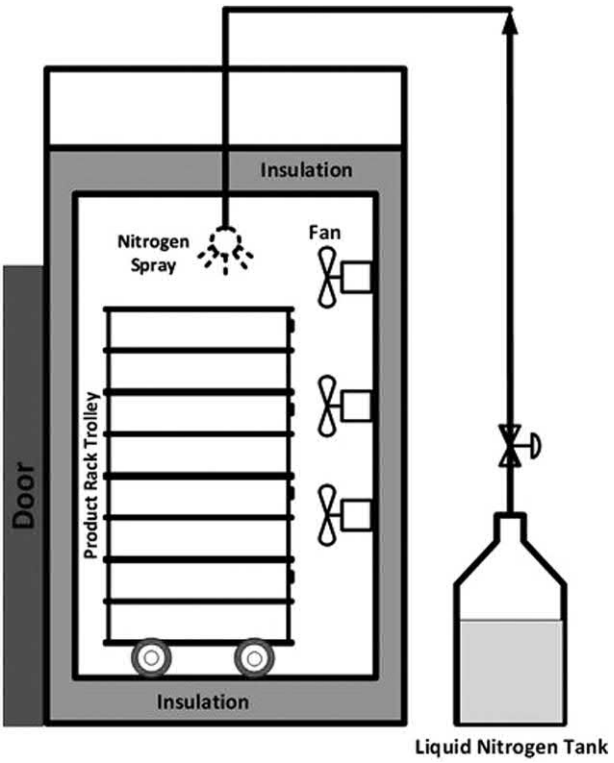

FIGURE 40.9 Cryogenic freezing in a cabinet.

–195.8°C [23]. Among the advantages of the cryogenic freezing method, we have a high freezing rate, low product loss (≤1%), easy handling, low initial and maintenance capital, low need for floor space, and excellent quality products.

Meziani et al. [25] conducted research to study the effect of immersion freezing with nitrogen on physical properties (structure and viscoelasticity) of dough, in which the freezing rate had a significant effect on rheological parameters for sweet doughs, with a higher reduction in elasticity (8.6 and 12%) as the temperature lowered (–30 and –40°C), attributed to the starch degradation and dough dehydration. Nagy et al. [26] completed a study on the effect of different freezing methods on selected properties of a pasta filata cheese using a slow freezer (i.e. static air at –18°C) and two rapid methods (i.e. blast at –30°C and cryogenic at –40°C). They found freezing times of 300, 75, and 50 min, respectively, to reach –12 for the first and –18°C for both rapid processes, resulting in easier-to-thaw cheese for the rapid methods than for the static air. Additionally, they followed other properties such as pH, texture, and sensory evaluation for the thawed food, and observed differences in favor of the quick freezing, which were influenced by the thawing method.

Diamante and Tran [27] compared three freezing methods: standard or static air (i.e. –15°C), blast air (i.e. –36°C), and cryogenic (i.e. –70°C). Although they did not find a significant difference in drip loss of beef meat, the average value decreased with the decrease in freezing temperature.

A cryomechanical freezing, or combined freezing with cryogenic followed by air-blast freezing, was utilized to improve frozen food quality. It is recommended for delicate products with poor mechanical resistance, such as shrimp, raspberries,

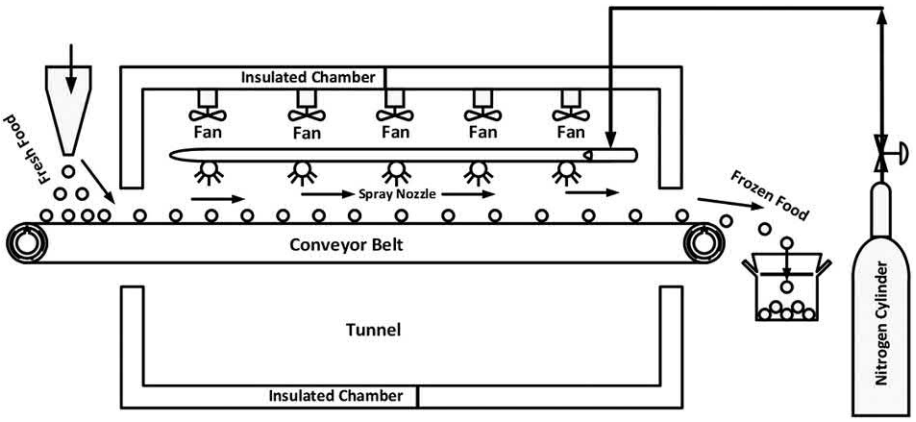

FIGURE 40.10 Cryogenic freezing on a conveyor belt.

and strawberries, or chicken and mushrooms when significant changes occurred in the product [28]. In this combined process, a protective crust formed through the immersion in the liquid nitrogen, characterized to the products.

Due to the importance of the freezing time, food engineers and plant designers need freezing equipment, and simple and accurate predictive mathematical models to calculate the freezing time. Therefore, several works related to modeling have been published in the literature [3, 17, 29–31].

40.5 EMERGING FREEZING TECHNIQUES AND FUTURE RESEARCH

Some new freezing techniques or combinations are being developed for their potential technical and economic advantages, as well as quality enhancements. High pressure and ultrasound, as non-thermal technologies, are being studied and used as pretreatments for foods that are subjected to freezing, in addition to other interesting ideas and variants. In particular, the freezing process and ice–water transitions may be affected by controlling pressure rather than temperature; the freezing point of water and the ice formed are functions of pressure [1].

Several kinds of high-pressure ices with different chemical structures and physical properties have been reported by Fletcher since 1970 and Hobbs in 1974. A pressure-shift or high-pressure freezing process can generate small and uniform ice crystals [32, 33]. Improved structures by pressure-shift freezing were reported for tofu (soybean curd) [34, 35] and carrots [36]. In the case of tofu pressure above 200 MPa, the texture was almost the same as the untreated tofu [34]. Similarly, in the case of carrots above a pressure of 100 MPa, the damage could be reduced significantly [36]. The freezing point of water can be shifted from 0°C at 101.3 kPa to –21°C under 210 MPa [33].

Zhu et al. [33] carried out a comparative study on pork muscle applying pressure-shift, air-blast, and liquid-immersion freezing methods. They found differences in size and distribution of ice crystal and consequent muscle damage, in which the pressure-shift freezing prevented the muscle disruption. Lately, Zhu et al. [37] completed another work, comparing the ice-crystal formation in a gelatin gel by pressure shift and conventional freezing (–20°C). At three pressure levels (100, 150, and 200 MPa), air freezing and immersion freezing were applied to samples, and the researchers observed a larger number of smaller ice crystals for those samples frozen at high pressures, with a better texture in the product.

A dehydro-freezing method is a combined method in which controlled dehydration is applied before the freezing stage; it is mainly promising for the preservation of fruits and vegetables due to their high moisture contents. This technique reduces refrigeration loads, and packaging, storage, and distribution cost, as well as providing a comparable quality to conventional freezing [32, 38]. Agnelli et al. [38] applied and modeled the dehydro-freezing of two fruits, pear disks and apple cubes, using glucose and sucrose solutions, before conventional freezing (air-blast tunnel at –40°C); they obtained good quantitative consistency between the experimental results and model predictions.

Ultrasound is a relatively new technology, and has been applied to some foods as a pretreatment of freezing; it has proven to be very useful for controlling both the nucleation and the crystallization growth processes, by generating nucleation sites. Sun and Li [7] also conducted comparative research on the freezing of fresh potato pieces of $17 \times 17 \times 76$ mm^3, based on microstructural observations of samples subjected or not to power ultrasound and then frozen by immersion in an ethylene glycol–water solution (50:50) at –18°C. They observed much less intercellular space and cell disruption. The freezing rate of potato samples was improved with the application of ultrasound in comparison with samples without this pretreatment. Magnificent cryo-SEM micrographs were obtained for samples exposed to both freezing variants, in which the plant tissue exhibited a better cellular structure and appearance for the potato subjected to power ultrasound. Ultrasound application in carrot juice for 30 min resulted in shorter freezing time for air and immersion freezing at –30°C, although the concentration level also affected the measured parameters. However, freezing by immersion was faster than with air [39].

Concepts based on engineering principles, mathematical knowledge, and modeling, as well as computer simulation,

TABLE 40.3
Engineering Concepts Significantly Related to Freezing Process, Frozen Storage, and Thawing

Engineering Concept	Comment	Tested Food	Reference
Freezing time	Combination of Plank and unsteady heat transfer equations	Beef	Mascheroni and Calvelo [43]
Freezing time	Comparison of existing approaches, analytical and numerical	Strawberries	Heldman and Lund [13]
Freezing and thawing times	Experimental and predicted freezing and thawing times	Lean beef	Cleland et al. [44]
Thermophysical properties	Computer program developed to predict freezing and thawing	Codfish	
Freezing and thawing times	Method based on enthalpy formulations	Peas	Mannapperuma and Singh [45]
Freezing and thawing times	Analytical method developed for high water foods	Fishes, meats	Salvadori and Mascheroni [46]
Properties, freezing time, and heat load	Prediction of enthalpy–temperature curves of foods over a range –40 to 40°C	Cheese, fish, fruits, meats	Pham [47]
Enthalpy and properties	Industrial processed food materials except fatty foods	Lean beef	Fikiin and Fikiin [48]
Weight loss kinetics	Mathematical model based on heat and mass balances and mass transfer phenomenon	Meat, potato Beef	Campanone et al. [49] Delgado and Sun [50]
Freezing and thawing times	Comparison of predictive models with experimental data	Fish, potato	Lopez-Leiva and Hallstrom [31]
Thermal-physical properties	Experimental and computer properties comparison	Green peas	Martins and Silva [51]
Heat transfer rates and enthalpy change	Numerical model based on energy equation with the Navier–Stokes equations	Beef, egg	Ho [52]
Quality loss kinetics	Computational evaluation of frozen quality profile	Green beans	Martins and Silva [53]

should be included as part of good process development, equipment design, and optimization of food freezers (Table 40.3). Quality kinetics need to be included in the optimization of the freezing process. The implementation of Hazard Analysis and Critical Control Point (HACCP), with all aspects of Good Manufacturing Practice (GMP), could be properly applied to ensure the quality and safety of frozen foods.

Finally, although the freezing process for foods and the existing options or methods have been revised and exposed, there are two very important aspects that should be emphasized in order to complete the production of an excellent food item, ready to eat. The first is the selection of an appropriate freezing process and the second is the correct storage of frozen food products; well-controlled commercial storage completes the good performance of the freezing process and results in high-quality products. The effects of thawing are often more damaging than the freezing itself. Therefore, not only is the right choice of the freezing method very important, the next two process stages, frozen storage and thawing, are necessary to achieve high-quality products.

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41 The Freezing–Melting Process in Liquid Food Concentration

Mohammad Shafiur Rahman, Mushtaque Ahmed, and X. Dong Chen

CONTENTS

41.1 INTRODUCTION

The freezing–melting (FM) process is capable of removing water by freezing it out from solution as ice crystals. Ideally, the ice formed should be free of solutes. First, the solution is partially frozen, and then the ice crystals are physically separated from the residual solution (i.e. concentrated), and finally, the ice is melted to form the product water. Ice crystals formed under the appropriate conditions can be very pure.

The freezing–melting (FM) process or freeze–concentration (FC) has the following advantages: (i) it has very low energy requirements as compared to those of distillation processes [1, 2] since the latent heat of fusion of ice is only one-seventh the latent heat of vaporization of water; (ii) it could lead to a 75 to 90% reduction of the energy required by conventional thermal processes [3]; the costs of concentration by evaporation and FM are listed in Table 41.1; (iii) it operates at low temperatures, which minimizes scaling and corrosion

TABLE 41.1
Comparing Operating Costs of Various Concentration Processes

Application	Cost per 1000 kg Water Removed	
	Evaporation	Freezing–Melting
Fruit juice concentration	$5.40	$1.98
Sugar production	$8.47	$1.32
Desalting seawater	$1.85*	$0.93
Caustic soda concentration	$2.23	$1.06
Black liquor concentration (for paper-pulp processing)	$3.15	$1.52

* This figure is now $0.84 in new plants in UAE.
Source: Chen [162].

problems [1, 4, 5], and inexpensive plastics or low-cost materials can be used at low temperatures [2, 4, 6, 7]. In the case of liquid foods, other advantages are (i) less thermal damage of the components, (ii) avoidance of off-flavor development, and (iii) minimal loss of volatile components. A very high surface area and high heat transfer coefficient can be achieved with direct contact between the brine and refrigerant. It has a general absence of pretreatment, chemicals required, and insensitivity of fouling and nature of solution [6, 8], and low ecological impact [7].

The use of FM is more common (instead of evaporation) in concentrating liquid foods due to the reduced loss of volatiles, aromas, color, nutrients, and thermal degradation of food products [9, 10]. The juice and dairy industries have used the technology successfully. It has been utilized commercially for the concentration of citrus fruit juices, for vinegar concentration, and for the concentration of beer and wines. This technology has also been used for concentrating coffee and tea extracts, sugar syrups, maple sugar syrups, dairy products such as milk and whey, and aroma extracts [11]. More common to the food industry are the indirect contact crystallizers, where the refrigeration energy must pass from the aqueous food liquid through the walls of an appropriate heat exchanger. The most common ones are the static layer growth system, layer crystallization on rotating drum, dynamic layer growth system, and suspension crystallization unit. Grenco [12] reported about 50 plants in commercial operation using the FM process in the food industry. Some commercial applications of FM processes in the food industry are listed in Table 41.2. The food industry has tailored the technology to their areas of applications and has taken advantage of the process. Sanchez et al. [13] reviewed the applications of the FM process in fruit juice industries.

The aroma and flavor of fresh pineapple and orange juices produced by FM were better preserved in their sensory qualities than when concentrated by thermal evaporation [9, 14]. There were no significant differences in the browning and turbidity of clarified pear juices (10°Brix) produced by vacuum evaporation (VE), reverse osmosis (RO), and FM and stored at refrigerated storage after 10 days [15]. Juices obtained by RO and FM showed a similar sensory quality, and these were superior to the EV. Similarly, single-strength clear pear juice (10°Brix) produced by VE, RO, and FM showed small differences in color, turbidity, heat stability, and sensory preference after storage for 10 days at 4°C [16].

Traditionally, the disadvantages of the FM process as compared to evaporation and reverse osmosis are its higher capital costs and operating costs during the ice separation [17]. Other disadvantages are (i) in the case of desalination, the retention of undesirable flavors and aromas (initially present in the feed saline water) that may come into the produced fresh water [9]; (ii) FM needs to include the growing, handling, and washing of ice crystals steps in case of desalination, and the need for mechanical vapor compressors; (iii) compressors represent an

TABLE 41.2
Applications of Commercial FM Systems for Food Liquids

Food Liquid	Product Concentration	System	References
Fruit juice	40~55wt%	Grenco in USA, Japan, Italy	Grenco [163], Muller [17], Deshpande et al. [23]
Vinegar	12.8~40wt% acid content	Girder in USA	Staff [164]
	up to 400-g	Votator	Votator [165, 166]
	48wt%	Grenco in USA	Wagner [167]
Beer and wine	12.5wt%	Phillips	Deshpande et al. [23]
	32% by volume	Grenco	Wagner [167]
	four-fold	Grenco in UK	Grenco [163]
Coffee extract	35~48wt%	Grenco in Brazil, Japan, UK, Switzerland	Grenco [163], Wagner [167]
Sugar solution	Up to 50wt%	Grenco	Wagner [167]
Whey	Up to 40wt%	CSI	Saal [168], Davis [169]
Skim milk	Up to 36wt%	Grenco	Deshpande et al. [23]. Wagner [167], Basta and Fouhy [170]
Whole milk	Up to 38wt%	Grenco	van Mil and Bouman [171], Basta and Fouhy [170]
Tea extract	Up to 35wt%	Grenco	Wagner [167]

expensive method of furnishing the energy requirements of the system [1]; (iv) probably the greatest deterrent to general acceptance of the freezing process is the fact that large plants cannot be designed and optimized with confidence, owing to the complexity of the unit operations in the freezing-unit, melting-unit, and wash-separation column; (v) in the case of desalination, trapping of salt solution in the ice during crystallization requires the crushing and re-crystallization of ice; (vi) a progressive increase in the concentrations of the dissolved substance, and non-condensable gases; (vii) high-quality energy is required for crystallization as compared to low-quality energy used in many evaporation processes; (viii) in the case of desalination, a certain amount of fresh water is required to wash ice so as to reduce salt content in the product water; and (ix) limited variations of the methods for the complete separation of ice from brine are available [18].

41.2 STATE-OF-THE-ART OF THE FREEZING–MELTING PROCESS

41.2.1 General Description

A refrigeration system is required to remove the heat of fusion of the ice from the solution. The quantity of heat which must be removed from the freezing-unit is essentially equal to that which must be added to the melting-unit [1, 6]. The FM process is accomplished in two major stages: ice crystallization (stage I), and separation and melting (stage II) (Figure 41.1). In stage I, nucleation occurs at a suitable supercooling temperature. The nuclei in solution grow to become large ice crystals in a crystallizing unit. In stage II, the crystals are separated from the concentrate by a separator (mechanical), and then the melting process is carried out to produce pure water or to use it to cool the warm solution.

In general, the components of FM process are (i) a *precooler* to cool the feed solution, (ii) a *crystallizing unit*, where sufficient heat is removed from the process fluid to crystallize up to 15% of the mass, and (iii) a *crystal separator and purifier*, where the crystal is separated from the unfrozen concentrate and washed with a few percent of the melted product to remove any adhering concentrate from the surface of the crystal. The separated concentrate is recycled to the freezer to provide any desired recovery, (iv) a *heat pump* that takes heat out of the freezer and transfers it either to cooling water or to the melted crystal (melting-unit) that is removed from the purification section, (v) a *feed heat exchanger*, usually employed to pre-cool the feed by using the cold product and the concentrate, thus reduces the load on the freezer.

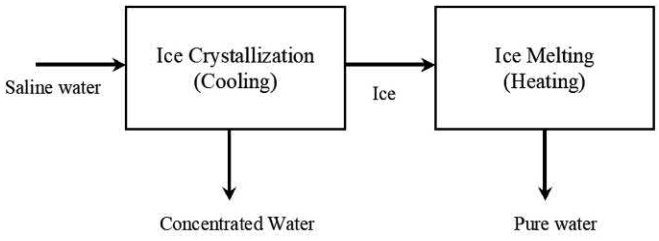

FIGURE 41.1 Freezing–melting process.

41.2.2 Historical Development

The Danish physician Thomas Bartholinus (1616–1680) was apparently the first to report that water obtained by melting of ice formed in seawater was fresh. Almost at the same time Robert Boyle (1627–1691) reported the same observation, foreseeing the phenomenon as a source of fresh water, and the Jesuit Athanasios Kircher (1602–1680) discussed the reason why ice formed in the sea is fresh [19]. The reason for getting fresh water from ice is due to the rejection of solutes at the interface. Freezing in large bodies of water occurs in nature on the surface of oceans, lakes, and bays. The combined heat transfer associated with heat removal by the environment and latent heat release at the water–ice interface result in natural convection flows of water. Freezing seawater releases fluid at the water–ice interface which is denser than the ambient water. The resulting solute buoyancy force therefore acts downward. This solute buoyancy force is in addition to the thermal buoyancy force. Flow visualization revealed that the flow was downward, below the freezing surface. Convection heat transfer rate is found to be strongly affected by solute rejection upon freezing [20]. At the end of the 18th century, the Italian scientist Anton Maria Lorgna (1735–1796) described a method to purify seawater and impure waters by freezing and then melting of the ice. In 1786, Lorgna published his first paper on water desalination by freezing, wondering why nobody had previously applied it in an artificial process initiating what nature does so well and easily in the cold seas: producing blocks of fresh water from ice produced in seawater. He also identified that only one single freezing of seawater produced an ice block with salinity, although much less than seawater salinity, thus a multi-stage freezing–melting process is required [19].

The method of water purification by FM was not of practical interest before the development of refrigeration machines. It was only possible in the coldest regions and seasons. The interest in the process for obtaining fresh water from seawater by freezing was revived in the late 1930s, and an experimental desalting plant has been operated for some years near Rome by the Istituto Superiore di Sanita. The plants operated by the indirect freezing process later revealed themselves to be of limited practical interest in comparison with the direct freezing desalination procedures. The FM process was first used commercially in the 1950s. Research in the 1960s and '70s for desalination, petroleum, and food processing applications provided many technical innovations [21].

41.2.3 Classification of the Freezing–Melting Process

Wide varieties of FM systems are currently on the market [22]. Further details of the different types are available in Heist [3], Deshpande et al. [23], Maguire [7], and Chowdhury [22]. In general, the FM process occurs in a crystallizer, although control of

TABLE 41.3
A Possible Classification of the Freezing–Melting Process

A. Direct-Contact Freezing
  a. Conventional direct-contact ice forming
  b. Gas hydrate method
  c. Eutectic freezing
B. Indirect-Contact Freezing
  a. Internally cooled
    1. Static layer growth system
    2. Layer crystallization unit on rotating drum
    3. Progressive crystallization unit
    4. Falling-film type
    5. Circular-tube type
    6. Suspension crystallization
  b. Externally cooled
    1. Vessel type (atmospheric)
    2. Vessel type (pressure shift)
    3. Spiral-finned type
C. Vacuum freezing
  a. Vapor compression system
  b. Vapor absorption
  c. High-pressure freezing and melting
  d. Vacuum freezing multiple phase transformation FM process
D. Hybrid System

ice formation and growth has been obtained in numerous ways. A classification of FM processes is given in Table 41.3 based on whether there is direct or indirect contact of refrigerant with the solution. Figure 41.2 shows the main difference between the direct and indirect methods of FM processes. In the case of direct FM, ice is formed within the solution, while in the case of indirect FM ice is formed on the cold metal surface, and refrigerant or coolant is not in contact with the solution.

41.3 DIRECT-CONTACT FREEZING

Direct-contact crystallizers provide for intimate mixing between the refrigerant (such as Freon and butane) and the product to be frozen. The refrigerant in liquid form under pressure is expanded through a nozzle into the product liquid, where it vaporizes at lower pressure. This vaporization provides a refrigeration effect and causes the formation of ice and/or solutes crystals within the product. A typical direct FM system (shown in Figure 41.3) is composed of an ice nucleation, a crystallizer allowing subsequent growth of these nuclei up to a size suitable for separation, an ice crystal separator, a washing-unit, and a melting-unit [5, 24].

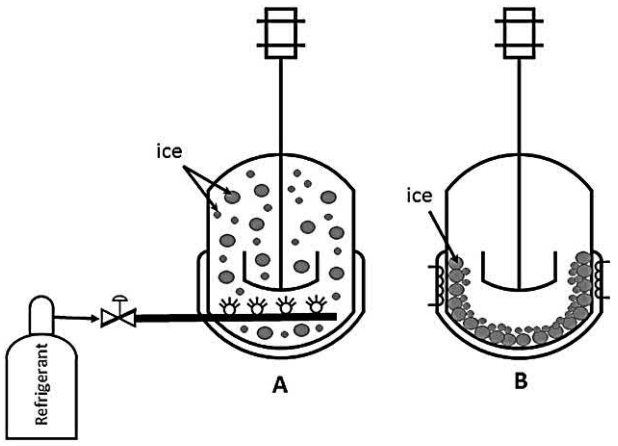

FIGURE 41.2 Two major methods of FM processes. A: Direct FM process, B: Indirect FM process.

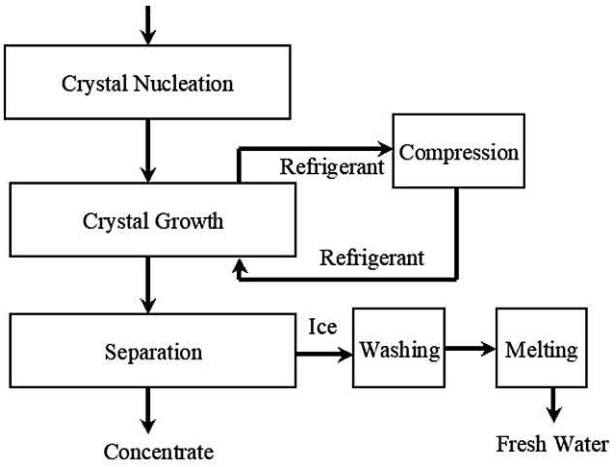

FIGURE 41.3 Schematic of a typical Direct-Contact FM Process. (From Hartel [5].)

41.3.1 Conventional Direct-Contact Ice Forming

A direct-contact freezer uses a spray of refrigerant by jet impact through a nozzle. The main advantages are: a high production rate per unit volume at a low driving force, power consumption is small, the absence of moving parts, and the unit is compact and efficient [25]. The successful design of a direct -contact FM plant significantly depends upon the availability of a suitable refrigerant [26].

There are certain thermodynamic, chemical, physical, and economic requirements, which the refrigerant must meet in order to be suitable for use in the process: (i) the refrigerant should have normal boiling point of –4°C or less and have a vapor pressure below 2.8×10^5 Pa at room temperature, (ii) the refrigerant should be nontoxic, preferably non-flammable, and chemically stable in solution, (iii) the fluid should be virtually immiscible with water and should possess such molecular size factors so as not to form a hydrate under the freezing conditions employed in the process, and (iv) the refrigerant should be cheap and readily available from commercial suppliers [27]. The details of thermodynamic properties of normal butane at refrigeration temperatures are compiled by Kurnik and Barduhn [28]. The refrigerants that could be used are butane, carbon dioxide, nitrous oxide, Freon 114, and Freon 318. Freon 114 and Freon 318 are better choices based on the above factors; however, these materials are relatively expensive when compared to other refrigerants such as butane. Antonelli [29] developed a process

whereby LNG evaporates and generates power, part of the seawater is frozen, and the ice produced is melted at ambient temperature.

It is possible to couple an FM process to an LNG vaporizer [30]. In many cases, gas companies import huge quantities of liquefied natural gas (LNG), which is vaporized from low temperatures to the ambient temperature at the terminals, and then transported through gas pipelines. In this case, seawater could be used as a source of heat for the vaporization of LNG. The cost could be substantially reduced if sea-water could be cooled from the ambient temperature to near freezing. The major operating parameters of LNG cold energy, such as coolant temperature, freezing duration, supercooling, seeding, agitation, crystallizer material, and subsequent washing procedure on ice production and water quality need to be optimized [31]. Theoretical analysis showed that the LNG-FM method consumed 1 kg equivalent LNG cold energy to produce 2 kg of ice-melted water, and the power consumption of this process was negligible [32].

Butane evaporation involves at least three phases, butane liquid, butane vapor, and liquid brine, and its mechanism is correspondingly complex. Simpson et al. [33] studied the evaporation process of refrigerant, describing the dynamics of the bubble motion in a more meaningful way, and recording more readily with the visual evidence of the bubble's motion. The rate of evaporation of butane droplets increased rapidly with diameter ratio and then gradually with one-sixth power, implying that evaporation was controlled by the heat transfer through the transient liquid butane film on the inside surface of the bubble [33].

Orcutt and Hale [34] used mathematical models to study the operational-design economics of a freezing process and to predict the best operating conditions. Optimization computations showed that the economics of process operation depend largely on the temperature maintained in the freezer and the overall difference in refrigerant and equilibrium freezing temperature. An analysis of the linearized freezer dynamic equations showed the freezer to be stable and did not indicate regions that were difficult to control. The cost of the washer-melting unit is influenced by the operation of the freezer, which determines the value of crystal size. The freezer operating costs depend on the brine temperature, which influences both the crystal size and the refrigerant vaporization rate.

The choice of a suitable refrigerant for the process is important. From the viewpoint of cost and stability, the hydrocarbons with four carbon atoms have been recommended. When n-butane is used, the operation is to be carried out carefully because it is at a vapor pressure lower than atmospheric pressure, while i-butane has a considerably higher vapor pressure than atmospheric pressure and can form solid hydrate in contact with the aqueous phase under specific conditions. Formation of hydrates results in the elimination of the ice crystals formed. The mixture with less than 73.8% i-butane cannot form a hydrate at –0.7°C with 1.6% sodium chloride aqueous solution, and this limiting ratio increases with increasing temperature [35].

41.3.1.1 Ice-Crystallization Unit

A direct FM process can be continuous or batch. Energy recovery is one of the important aspects of the process. A butane FM process employs butane as the immiscible refrigerant (Figure 41.4). The refrigerant in liquid form under pressure is expanded through a nozzle into the product liquid, where it boils at lower pressure. The vaporization of butane in the freezer removes heat from the brine, causing a portion of it to freeze as tiny ice crystals (Figure 41.5). Another option is to use a high vacuum to vaporize a portion of the water, which then provides the refrigeration effect for lowering the temperature of the product and causing ice crystallization to occur. This process is able to reduce the residence time in the crystallization unit at least half compared to flash freezing [26]. These types of processes are mainly utilized for the concentration of chemicals and seawater desalination [1, 36]. It is rarely used in the food industry for a number of reasons. The major reason for not using direct-contact freezers in food concentration is that a vapor–liquid interface is created, resulting in a subsequent loss of volatile flavors and aromas. The product then has no superior quality advantages over that produced by evaporation [5].

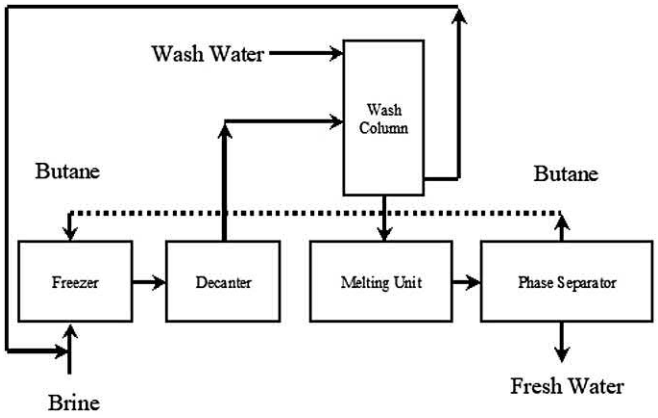

FIGURE 41.4 Simplified flow diagram of a butane FM process.

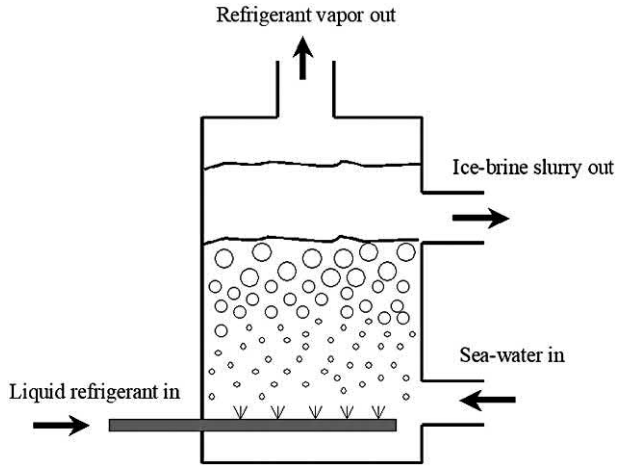

FIGURE 41.5 Schematic diagram of a direct freezing unit. (From Rice and Chau [55].)

The ice nucleation unit produces small ice crystals, which are transferred into the crystallization unit and grown large by ripening (i.e. larger in size) at the expense of smaller ones. In the crystallization unit, the formation and growth of ice crystals should be controlled in such a way that a uniform distribution of large ice crystals suitable for separation is formed. The optimum size distribution for most separators is a monodisperse distribution (narrow range of sizes) with a large mean size. This facilitates the washing step and reduces the amount of product carryover into the separated ice stream. The more efficient the separation process, the less carryover of solute into the separated ice stream, and the more economical the overall process is.

The ice crystals are then collected and transferred to the ice crystal separator and washed with water to remove the brine or solution from the ice crystal surface. The key technology in this system is how to grow ice crystals in the crystallization unit large enough to facilitate the separation between the ice crystals and solutes [37]. From a separation point of view, the formation of a few large ice crystals is desirable. Velocity of nucleation and rate of growth depended on the supercooling and concentration [38, 39]. In highly concentrated sucrose solutions, the nucleation rate did not depend on concentration, and the rate of crystal growth declined due to increase in viscosity [38].

Supercooling and secondary nucleation were identified to be major factors preventing ice crystals from growing large [40–42]. Very high supercooling can create a large number of smaller new crystals. Lower rates of supercooling are, therefore, desirable to prevent excessive nucleation. Lower nucleation rates are required to produce reasonably large ice crystals at an acceptable residence time [23]. The crystal growth at high supercooling occurred dendritic- or needle-shaped, which raised a larger surface area and caused difficulty of separation from concentrated solution.

Thijssen [39, 43] suggested increasing the agitation rates, within certain limits, to lower the nucleation rates, since high mixing rates may promote smaller size due to mechanical damage. Garabedian and Strickland-Constable [44] found that fluid shear does not produce crystal breeding, and collision of a single crystal in pure water produces high rates of nucleation. Polycrystals may well be formed by agglomeration or growing together of fine crystals. The nucleation rate at low stirring rates is determined primarily by the cooling rate, while with intensive stirring it depends primarily on the hydraulic factor [45]. Polymers could suppress the secondary nucleation of ice crystals; thus larger crystal size could be achieved. The suppression depends on the types of polymer and concentration, and is related to the increase of viscosity [42].

The circulation pattern of the ice slurry in the freezing-unit by direct-contact vaporization of an immiscible refrigerant can profoundly affect the quality of the product crystals [46]. Stripping of butane from products has been accomplished in a packed tower with liquid effluents containing less than 0.2 ppm butane which meets some standard for desalinated water [26]. Landau and Martindale [36] reported initial bench-scale studies of novel butane freezers, the most promising of which used a draft tube. The butane introduced at the bottom gave good vertical movement to the slurry, and 25% ice suspensions could be handled. It was found quite unnecessary to use a mechanical agitator, and the unit operates satisfactorily without any additional agitation, and a comparatively small flow of butane vapor, purged in near the bottom of the crystallizer, greatly improves the mixing and ensures reliable operation under all circumstances. This small flow of vapor (containing a negligible amount of incondensable gas and little super heat) is still effective in causing circulation even when the pressure in the bottom half of the crystallizer is above the vapor pressure of the butane [47].

Barduhn [26] concluded that the following points should be considered in designing secondary refrigerant freezers: (i) adequate dispersion of the liquid refrigerant into the solution is of paramount importance, (ii) normal butane is the cheapest and probably best refrigerant and does not hydrate, (iii) short residence time does not necessarily lead to small crystals and poor washability, (iv) the plant should be designed to handle short contact times, and (v) several methods of agitation should be included in design.

Some of the best possible ways of mixing could be (i) the use of fine spray nozzles to introduce the liquid refrigerant under the brine, (ii) pumping vapor from the vapor space through spargers which reintroduces it under the brine, (iii) pumping the entire liquid content of the freezer rapidly around a closed path, and (iv) using conventional mechanical agitators. The latter method is the most difficult to scale up, and furthermore multiple mixers appear to complicate the design and increase costs in large plants. The combination of (i) and (ii) could be a viable option [26].

The ice production rates in a spray freezer can be 10 to 30 times those in stirred tanks. Refrigerants and saltwater are sprayed into a low-pressure space and slurry forms virtually instantaneously, but the particle size averages only 40 microns and the ΔT (temperature gradient between refrigerant and slurry) is very large, 18°C [48]. In the case of RC-318 (C_4F_8) the ice crystal size was a strong function of the salinity, and a marked size maximum occurred at about 0.5% NaCl. A similar phenomenon is also noted in single crystal growth rates from many aqueous solutions [49]. At least two factors, namely diffusion and surface adsorption, could be rate controlling in a continuous crystallizing unit [50]. Depending on the liquid depth and temperature conditions in the freezer, several liquid refrigerant zones may exist [51]. Refrigerant at depths sufficient to suppress vaporization is said to be in the *inactive* zone. Vaporizing refrigerant is in the *active* zone, and if liquid refrigerant accumulates on the surface of the slurry, it is said to form an *excess* zone. Nucleation mainly occurs in the active zone, while crystal growth proceeds throughout the entire brine. The depth of the active zone can be calculated from the relationship between the refrigerant vapor pressure and temperature. It is good to prevent the formation of an excess zone, which generally interferes with good freezer control [34].

The exchange FM process was developed by Johnson et al. [49]. The crystallizing unit is a horizontal vessel, operating

at atmospheric pressure, consisting of three distinct sections: (i) an ice-brine and hydrocarbon disengaging section, (ii) an agitated or contacting section, and (iii) a brine and hydrocarbon disengaging section. The brine entering the agitated section is broken up into small droplets by turbine agitators and counter-currently contacted with a partially solidified stream of normal straight-chain hydrocarbons. The melted hydrocarbon, which contains entrained brine, flows into the disengaging section where separation is effected by gravity and electrostatic coalescence. The electrical coalescer is a horizontal unit consisting of three vertically stacked grids.

Homogeneous nucleation of ice crystals requires a subcooling of several degrees and does not occur in the direct-contact freezing process for which brine temperatures are much closer to the freezing point. All new crystals are formed from existing crystals by secondary nucleation processes such as breaking of crystals by collisions or removal of fragile dendrites by fluid shear [46].

Applying ultrasound to crystallizing systems offers significant potential for modifying and improving the process. The most important mechanism by which ultrasonic can influence crystallization is ultrasonic cavitation, which is particularly effective for inducing nucleation. Using ultrasound to generate nuclei in a relatively reproducible way offers a well-defined starting point for the crystallization process, and allows the focus to be on controlling the crystal growth for the remainder of the residence time in the crystallizing unit. This approach can successfully manipulate crystal size-distribution and hence modify solid/liquid separation behavior, washing, and fresh water purity [52].

The performance of suspension growth systems can be improved by adopting a multi-stage design [12]. The main advantages of the multi-stage plants include lower energy consumption and approximately 50 to 70% lower operating costs than the single-stage process. The loss of soluble solids usually is less than 100 ppm after the washing step in the Grenco systems.

Water from the direct refrigerant FM process would contain excessive amounts of volatile refrigerant, which is undesirable in most cases. This process could retain 80 to 140 ppm of butane, and up to 3% CH_2ClF, CF_2Cl_2, or other halogenated hydrocarbons [53]. These gases must be recovered and recycled down to about 1 to 10 ppm to recover their economic value and/or to prevent explosion hazards, and probably to the 0.1 ppm range to meet public health standards for drinking water [1, 54]. When the dissolved gas is to be recovered after removal, it is important that the process be uncomplicated in order to keep the cost low. Simple flashing of the product streams at reduced pressures is useful, and probably necessary, but not sufficient since the approach to equilibrium in a single-stage flash is not very close because of the very short residence times in spray chambers. In this case, lowering the flashing pressure is the key to maintaining the low ppm of the refrigerants. Bajolle et al. [54] designed a method for stripping butane from water in a packed column down to concentrations as low as 0.6 ppm. The mass transfer of butane from the melted water was experimentally shown to be liquid-diffusion controlled. The results obtained are expected to be valid as long as the thermal effects are not significant, that is, as long as the column is operated at a total pressure not below the vapor pressure of water.

The refrigerant compressor limitations and problems are some of the important limitations for a direct FM process. However, they can be eliminated by the use of a new type of compressor known as the hydraulic refrigerant compressor [55]. The hydraulic refrigerant compressor does not use lubrication (i.e. avoids lubricant contamination), and water and water vapor carried into the compressor inlet have no detrimental effect on the compressor. This compressor is highly efficient; thus it avoids the problem of freeze desalination caused by conventional compressors. At the freezer and melting-unit temperatures, the pressure of the two-phase n-butane refrigerants is very near atmospheric pressure, which is a large advantage in the FM system since the pressure vessels need only to withstand very small pressure differences and can be of minimal strength and cost. The advanced technical skills associated with the design, installation, and maintenance of conventional refrigerant compressors are not needed, and parts are inexpensive. The hydraulic refrigerant compressor is simple, of low cost and low maintenance. It is compatible with the other components of an FM process.

41.3.1.2 Ice Separation

Separation devices can be classified as presses, gravity drainage, centrifuges, filter, and wash columns [39, 56–59]. Filtration has proven less effective for crystal separation, and it cannot be used for washing the crystals. In many cases, screens or filters show a history of freezing up (brine freezing in the openings of the weave) [3]. Arulampalam et al. [60] investigated the effect of various physical parameters on the efficiency of separation and purification. The important factors controlling the separation efficiency of the columns were the axial diffusion of impurity and the mass transfer between the adhering and free liquids around the crystal phase. Modification of the screw conveyor was necessary to facilitate crystal removal. Conventional methods used for the separation of crystals from their mother-liquid prove to be either too slow or too expensive [61].

41.3.1.3 Wash Columns

In the case of an FM process, washing is important to attain certain purity. In the case of a food solution, washing could recover the adhered solution from ice. After the crystals are formed in the crystallizing unit, the crystal/liquid slurry is separated into concentrated liquid and other crystalline components and impurities are washed from the crystal surface, producing pure crystals. To perform the separation, a wash column is used. There are two types of wash column: pressurized and gravity. In the pressurized wash column, the crystals rise to the top and hydraulic pressure forces a wash liquid, derived from the melted pure crystals, to flow down. The applied pressure also squeezes the concentrate through a filter at the bottom of the column. As the wash liquid flows down the column, it removes impurities from the surface of

the crystals. At the interface between washed and unwashed crystals, called the wash front, the wash liquid comes in contact with colder crystals and crystallizes on them. In this way, the wash liquid doesn't mix with the concentrated liquid. Effective washing of ice is one of the most difficult unit operations in the FM process.

The gravity wash column is simpler in design but larger than the pressurized wash column. Its greater height creates the pressure needed to compact the ice bed. It works in much the same way as the pressurized column but at lower pressures. An ice pack is still formed and moved hydraulically up the column. The performance of wash columns depends on the crystal size and shape and on the viscosity of the mother-liquid. Uniformity of crystal size and shape is important to avoid having the wash water seek the path of least resistance and channel through the crystals unevenly.

In order to overcome the difficulties associated with surface tension forces, clean fresh water can serve as a displacing liquid. The displacement process can best be pictured by considering a gravity drainage separation process. The separation by drainage is greatly improved when in addition to draining the brine from the bottom of a batch of ice crystals one adds pure water to the top of the batch and lets water filter through the interstices of the ice bed to displace the remnants of brine. Bosworth et al. [62] suggested a continuously operating separation device based on the displacement principle. In this device, the slurry of brine and ice crystals is introduced into the bottom of a vertical column from which the brine drains through screens at the bottom. The ice crystals move upward by their own buoyancy force forming a porous ice plug at the top of the column where wash water is added from the top. As the ice plug moves upward through the layer of wash water the brine is displaced from the interstices of the porous ice plug and the salt-free ice crystals are harvested at the top of the column and transferred to the melting-unit. The rise of the ice crystals in the column is, however, rather slow, being a limiting velocity for a particle moving through a fluid by buoyancy (or gravity) forces alone. This limiting velocity determines an upper limit to the production rate for such a gravity wash column, a value that is much too low for economic desalinated water conversion. Hahn et al. [63] modified the wash column separator described above to allow the moving brine to provide the driving force for moving the ice particles upward. The discharge screens are located in the vertical walls of the column, about midway between the top and bottom.

Wash columns have been successfully used for both separating the crystals produced in the crystallization section as well as purifying them. These are classified as the flooded column and the drained column. The ice crystal slurry is fed to one end of the column, and the mother-liquor withdrawn from the same side. The compacted ice bed is forced towards the other end of the column, where it is melted by means of a grid of internally heated pipes. The portion of meltwater is used to wash the crystals moving counter-currently in the column [23].

The ice wash column could be a drained or flooded type with either a rectangular or cylindrical shape. Tall screens are desirable, and contouring of the ice surface with the scraper can be very useful. Much higher production rates were possible by cutting out a slot in the center of the column, which fills with wash water, or by cutting a deep V-shaped trough in the ice with the scraper [26]. In a typical hydraulic piston washer-separator column, the ice–brine slurry is fed to the bottom of the column and moves upward. The ice builds up a porous plug until it fills the entire top of the column; the brine flows out of the column through filter screens situated laterally part way up the column walls. As slurry is fed to the column, ice is added to the bottom of the porous plug and the brine flows through the plug. The pressure drop of the brine flowing through the plug causes the ice to move upward, and it is harvested at the top of the column, usually by scraping. The washed ice is melted, and a portion is recycled to the top of the washer separator as wash water. This wash water moves downward by gravity, and some of it leaves the column through the filter screens and the brine from the slurry, but the greater part of the wash water clings to the washed ice [34, 61].

The production of fresh water increases with the increase of ice crystal size, the ice plug length above the screens, concentration of ice crystals in the slurry, and the external mechanical restraining forces on the ice plug. It is also found to increase with a decrease in the ice plug length below the screens, where this length is shown to be an independent parameter for certain slurry feed conditions, such as gravity feed. Shwartz and Probstein [61] provided design criteria for minimizing the capital cost for a given production rate. The theoretical analysis helped to establish the optimal operating conditions for high production rates, sufficiently low salt content in the product, and minimum loss of fresh water. At pressures below about 1.05×10^5 Pa, the driving force in the melting-unit becomes so low that the required melting-unit cross-sectional area increases to large values, which are uneconomical [34].

Centrifuges have also been used to separate the ice crystals from the concentrated liquid. Filtering centrifuges utilize the difference in specific gravity between ice crystal and liquid concentrate to separate ice from liquid with the liquid being forced through the filter basket by centrifugal force. Generally, a water rinsing is required to assure that there is no salt carryover. For food liquids, there is some loss of volatile flavors and aromas due to the airspace created during the separation. It is important to have large ice crystals to ease the ice-crystal separation from the concentrated solution phase [64–66].

Ice particles from liquids during the FM process can be separated by a filter. To increase efficiency, a vacuum is normally applied to pull liquid across the filter. The vacuum filter has been used for separating ice slurry made by direct freezing and vacuum FM systems [67–69]. Solids carryover is one of the problems for the wash column; however, the Grenco system for separation can give up to 100 ppm purity [70]. This wash column operates continuously with counter-current flow of ice and concentrate. The ice crystals are transported to the upper levels of a vertical column by some natural or applied force, where they are then separated by a scraping device.

A small portion of the ice is melted and allowed to pass back down the column, giving the washing effect. Concentrate is removed through a filter at the bottom of the column.

The slurry of the ice crystals in brine is pumped to a wash separation column, where the brine and the ice crystals are separated with the consumption of a small amount of wash water. The washed ice is then sent to the melter-condenser, where it is melted by direct-contact condensation of the compressed butane vapor. The primary compressor must compress the butane vapor in order to attain its condensation temperature in between the brine freezing point and ice melting point. The two liquid phases formed in the melting-condensing unit are separated by a decanter, the liquid butane being returned to the freezer and the liquid water representing the product, except for a small amount which is used as wash water and leaves the wash-separation column with the reject brine stream. The net wash water consumption is about 2% [1].

The forced transport wash column was preferred over the gravity column due to its smaller size and short residence time, which produced relatively pure crystals [71]. In fruit juice production, the most common column used is the piston-type wash column in counter-current washing [72].

41.3.1.4 Melting-Unit

The melting-unit is difficult to characterize although it appears to be a straightforward process. Direct contact melting is easy to achieve; however, scaling up is difficult. In the case of a dumped bed, the characterization of heat transfer data is difficult due to the drainage of water and refrigerant from the bed. An indirect melting-unit requires a heat transfer surface, which reduces the attainable efficiency when compared with the direct-contact melting-unit [6]. A heat pump-operated FM system with a tubular heat exchanger was developed [73]. It selectively produced frozen water from seawater in the evaporator and melted the ice in the subsequent phase when it served as a condenser. The condenser optimally utilized the latent heat of melting of ice to condense partially the refrigerant, and the excess heat is rejected to the ambient environment, and the need for ice scraper/separation mechanisms is avoided. Attia [74] also proposed a similar system by utilizing the heat flow of a heat pump system to increase the whole system efficiency. Ice washing and melting process occurred at the same place of formation by reversing refrigerant flow through the vapor compression cycle so there is no need for ice-handing mechanical systems.

Lloyd [75] applied the concept of integration in order to achieve high utilization of space and to permit adjacent processes to share boundaries. A commercial design using two integral vessels for four main processing steps has been formulated. It showed that capital cost could be reduced by around 20%. Different commercial equipment is available based on this concept.

41.3.1.5 Refrigerant–Brine Interactions

The refrigerant is contaminated by brine spray carryover from the crystallizing unit, which adversely affects compressor performance. Thus, it is necessary to develop separating devices between the crystallizing unit and compressor. These separators must remove ice containing an evaporating refrigerant; thus simple demisters are not permissible because of ice plugging problems attendant to this condition. The dissolved refrigerant is rather easily removed by vacuum stripping of the effluent stream, which can be polished to meet environmental effluent standards by carbon absorption if necessary.

It is important to make certain that during operation of the process, excessive losses of refrigerant do not occur as a result of entrainment and solubility of Freon in the water, and from irreversible processes such as hydrolysis. In addition, if the rate of hydrolysis of the Freon refrigerant is too high, undesirable levels of soluble fluoride could build up in the product water. Stepakoff and Modica [27] developed the solubility data of Freon in order to predict the rate of hydrolysis, which is a function of dissolved Freon in water. They found the economic loss of hydrolysis is less than 0.26c per m^3 water. The solubility of refrigerants decreases with the increase of salinity termed as salting out. The hydrolysis rate was decreased to half for a 3% saline solution relative to pure water at the same temperature and pressure. Completely halogenated hydrocarbons are much less soluble than their partially halogenated counterparts. For example, Freon 142b (CH_3CClF_2) is ten times as soluble as Freon 114 at 1 atm, –4°C. The solubility of Freon 14 (CF_4) is about three orders of magnitude less than that of Freon 23 (CHF_3). The fact that hydrogen-containing Freons are much more soluble than their completely halogenated counterparts can be understood on the basis of hydrogen bonding between the CH group in the halocarbon and the oxygen dipole of the water molecule [76].

The simplest mechanism for describing the hydrolysis of Freon is to assume that the rate-determining step is a slow ionization to a carbonium ion and a halide ion followed by a faster reaction of the carbonium ion with dipolar water molecules [77]. The essential feature of this mechanism is that every carbonium ion which is formed in the primary step is attacked by water at a much faster rate than by halide ions, i.e. the reverse rate of the primary step is very slow. In the case of Freon 31 the mechanism of hydrolysis can be described by the following equations [78]:

$$CH_2ClF \rightarrow CH_2F^+ + Cl^- \qquad \text{(slow)}$$

$$CH_2F^+ + H_2O \rightarrow CH_2FOH + H^+ \qquad \text{(fast)}$$

$$CH_2FOH \rightarrow HCHO + HF \qquad \text{(fast)}$$

The presence of formaldehyde (HCHO) in the hydrolysis of Freon 31 was confirmed by chemical tests, and the rates of formation of chloride and fluoride were found to be identical [78]. Stepakoff and Modica [27] pointed out that a similar effect could occur during the hydrolysis of Freon 114. Stepakoff and Modica [79] determined the hydrolysis rate constants for Freon 21 ($CHCl_2F$), Freon 31 (CH_2ClF), and Freon 114 ($CClF_2CClF_2$) based on the three adjustable parameters, the Arrhenius collision frequency, hydration energy of the carbonium ion, and the ionic distance of closest approach.

The hydrates formation consisted of two phenomena, which were almost always observed when the freezer was being tested at low driving forces. First, the ice becomes oily, somewhat impalpable, and washing becomes poor. Operators called this rotten ice. Second, the level of butane in the storage tanks dropped steadily. This led to the accumulation of butane in the freezer, washer, or melter, and this could be due to hydrate formation. Insufficient agitation at low-temperature gradient can lead to accumulation of the liquid butane in the freezer, and it will then carry over into the washer, reducing the porosity of the ice bed, thus resulting in poor washing. In some cases, when refrigerant is well-dispersed, it shows no hydrate formation even with 80/20 mixtures of iso- and n-butane. Mixed refrigerants for which the vapor–liquid equilibrium is normal (i.e. the temperature–composition diagram is lens-shaped) have a disadvantage since the bubble and dew points differ. In the case of iso- and n-butane this difference between the compositions used is about 1°F. Thus 1°F is added to the total temperature lift of the heat pumping cycle, which adds about 10% to the energy requirement for primary compression beyond what is needed for a pure or an azeotropic refrigerant. Hydrates may also cause channeling in the wash column.

Hydrate formation is one of the problems for the direct FM process. The rate of heat transfer among the four phases present in a butane freezer controls the rate of ice production, and this in turn is determined mainly by the liquid–liquid interfacial area and the intensity of turbulence in the freezer.

The formation of hydrates is one of the problems that occur in the direct freezing process. Iso- and *n*-butane may form hydrates. Having two crystal species present (ice and hydrate) is uneconomical for the process since heat must be removed at the lower of the two formation temperatures and rejected at the higher of the two. In the case of ice and iso-butane hydrate this increases the total temperature lift for the primary compressor by about 1.9°C, which may increase the energy requirement by 30%. Even more serious than this is the fact that the hydrate reduces substantially the permeability of the crystal bed in the wash column. Butane-1 usually shows less hydration compared to normal butane. Operation on butane-1 was similar to that with the butane mixture, and hydrates and rotten ice still appeared under certain conditions. In addition, the smell of the commercial hydrocarbon bothers many people, and it costs about 31c per gallon as compared to 11c for normal and 14c for iso-butane [26]. Fernandez et al. [80] found similar rates of formation in hydrates in the case of propane, F-12 (CCl_2F_2), and methyl bromide (CH_3Br). Roux [81] found that F-31 (CH_2ClF) formed hydrate six to ten times as fast as the above refrigerants. The F-31 hydrate crystals were compact rough spheres with average diameters of 150 to 190 microns. Both propane and methyl bromide hydrates are smaller and much more dendrite-type and probably more compressible in beds. Hydrate properties depend on the type of refrigerants.

41.3.2 Gas Hydrate Method

Gas hydrates are crystalline complexes composed of water and gas molecules. A hydrate-based desalination process was offered in the 1940s [82]. In this process, hydrate is formed first to remove water from the solutions and then dissociated to water and gas. Fakharian et al. [82] developed a three-stage gas hydrate method for desalinated water using natural gas. First, hydrate was formed and concentrated solution was extracted from the reactor. The hydrate was washed with a suitable amount of fresh water to successfully improve desalination efficiency, and hydrate was dissociated to produce fresh water.

41.3.3 Eutectic Freezing

The eutectic FM process was first proposed at Syracuse University. Barduhn [83] devised the process, and Pangborn [84] tested the idea. In the eutectic FM process, salts separate as solids and fresh water from brine as ice. By freezing the water out of these aqueous solutions until they are adequately concentrated to precipitate the salt simultaneously, one ends up with no brine product. The ice and salt crystals nucleate and grow independently and are easily separated since the ice floats and the salt sinks. Using a simple system of sodium chloride and water the essential feature is that at –20°C both ice and $NaCl.2H_2O$ crystals precipitate from solution as heat is removed. Laboratory investigation of the eutectic temperatures of various proportions of the ions commonly found in natural waters (Na, K, Ca, Mg, Cl, SO_4, HCO_3) shows that the process operated well at temperatures no lower than –25°C [85]. This approach could avoid the brine disposal problem as well as the production of byproducts as salts. The main difference between this and the normal freezing process is the presence of the ice–salt separator and the salt filter. Several important variations of this have been proposed.

There is another important difference between normal and eutectic freezing in the crystallizing unit. In normal freezing where the product water is the only goal, the freezer operates near –5°C, and the temperature lift for the primary compressor is thus about 6°C. Two-stage eutectic FM is more economical [8, 85, 86]. The costs are generally much lower than the alternative of reverse osmosis followed by deep-well injection of the reject brine. Actually, deep-well injection could be ecologically unsound. The eutectic FM process may be a meaningful solution to brine disposal [86]. Schroeder et al. [8] pointed out that the eutectic FM processes are likely to become very important processes in the future when they are better developed. A stirred-tank crystallizing unit is required, and a hydro-cyclone separator and floating wash column are used [86]. The hydro-cyclone splits the slurry into a light and heavy cut. The light cut, called the overflow, contains the ice crystals and brine. The heavy cut, called the underflow, contains the salt solids and brine. The salt solids could be separated from the brine, utilizing conventional solid–liquid separators such as filters, and then dried in conventional dryers. The brine filtrate is required in the crystallizer [8].

Hydro-cyclones are compact, simple devices, and have a low pressure drop. Washing is different due to the small ice crystal size, and low slurry temperature. Schroeder et al. [8] identified the possibility of the combination of eutectic freeze

and distillation process, and membrane and eutectic freeze processes. The product stream of eutectic freezing would be a brine of low salinity, which would be returned to the distillation plant or membrane process in actual operation. The cost of the membrane process extremely depends on the brine concentration. The melted ice water from a single freezing without a wash step has three to six times less salt than the feed [87]. Usually a conventional direct-contact FM process operates around –5°C, whereas the eutectic FM process operates at –25°C, thus needing more energy for cooling. The main advantage of the eutectic FM process is that it can separate both ice and salts at the same time and avoids the brine disposal problems. For the precipitate salts, it could also be possible to produce different byproducts.

41.4 INDIRECT-CONTACT FREEZERS

In the indirect-contact FM process, the energy for refrigeration must be passed through the walls of some form of heat exchanger, and heat transfer occurs through a solid barrier [59, 88]. It was found that the growth rate of dendrite-type ice in supercooled water cannot completely be understood on the basis of the heat-flow controlled-growth mechanism but has to be explained on the basis of the combined mechanisms of heat dissipation and molecular-growth kinetics [89, 90]. Indirect-contact systems can be classified into those that are internally cooled and those that are externally cooled.

41.4.1 Internally Cooled

Internally cooled crystallizers can be further subdivided into static layer growth system, layer crystallization unit on rotating drum, progressive crystallization unit, dynamic layer growth system, and suspension crystallization processes.

41.4.1.1 Static Layer Growth System

In layer growth systems, the liquid from which the crystal mass is grown is stagnant, and it is termed static layer crystallization. The static operation of solution crystallization in this process is very reliable and requires very simple equipment without moving parts and without the need for a solid–liquid separation device. The residence time in this process is large because the mass transfer is only promoted by free convection. Large equipment volumes are required due to the batch-wise operation and the slow crystallization rate [91, 92]. The crystal–solution interface per unit equipment volume can be increased by using a plate-type contact surface, but static growth cannot be avoided [93]. High purification efficiency can only be obtained when relative low rates of growth ($<10^{-7}$ m/s) are established since no stirring is applied. The capital cost of the equipment is high, and more economic use can be obtained by carrying out more than one relatively rapid crystallization in series.

Muller and Sekoulov [94] pointed out that the layer freezing process is easier to manage, but the crystal growth upon a cooled surface induces fast crystallization rates, and under these conditions, a rather impure crystal film may be produced. In spite of this moderate crystal purity, the disadvantages are compensated for by the ease of operation, because there are no moving parts and no slurry handling is required. Two mechanisms of crystal growth were found in a batch crystallizer with an external cooler, that contained a large quantity of ice crystals. With the first mechanism, the ice crystals grew larger by the usual kind of growth, governed by heat or mass transfer resistance, and with the second, the ice crystals agglomerated and the agglomerate fused into a very large ice crystal (~1–3 mm in diameter). The second mechanisms occurred not because of the high concentration of ice crystals in the crystallizer but because of long residence time. Large ice crystal agglomerates were not produced when extremely small ice crystals were formed in the crystallizer at the start [66].

41.4.1.2 Layer Crystallization Unit on Rotating Drum

In the layer crystallization with rotating drum FM process, ice forms in thin layers on the heat exchange surface and, after a suitable period of time for the ice layer to build up, is removed from the surface and separated by press from the concentrated liquid remaining. One form of layer crystallizer utilizes a rotating drum immersed in the fluid to be concentrated. Refrigerant is circulated within the drum and causes ice to form on the surface of the drum, which is then scraped free as the drum rotates past a knife [5].

41.4.1.3 Progressive Crystallization Unit

A progressive crystallization FM process is a method of separating solvent from solution based on a concept completely different from the conventional method of layer crystallization described above [95]. A progressive FM process utilizes the concentration phenomena of a solute at the ice–solution interface moving from one end of a vessel to the other end [96]. It is characterized by having only a single ice crystal in the system so that the separation of the ice crystal from the concentrated solution is very easy compared with the conventional method of freezing–melting (Figure 41.6).

The concentration efficiency in progressive FM was related to the ice structure of the freezing front. High FM efficiency was obtained under the conditions at which a smooth solid–liquid interface is formed. The distribution coefficient depends on the ice structure at the freezing front, and operating conditions represented by the moving speed of the freezing front and the speed of stirring in the solution phase [95, 97]. Liu et al. [37] studied the operating conditions of this process on the freeze–concentration ratio and apparent partition coefficient of a solute between the ice and the solution phases (glucose and/or blue dextran). They found that the efficiency of the process is strongly dependent on the moving speed of the freezing front and the agitation or stirring speed at the ice–solution interface. A lower speed of the freezing front and a higher stirring speed produced a better freeze–concentration ratio. A concentration polarization model was useful to describe the effects of the operating conditions on the effective partitioning coefficient [97].

Matsuda and Kawasaki [98] investigated the effects of supersonic radiation and the dissolved air concentration

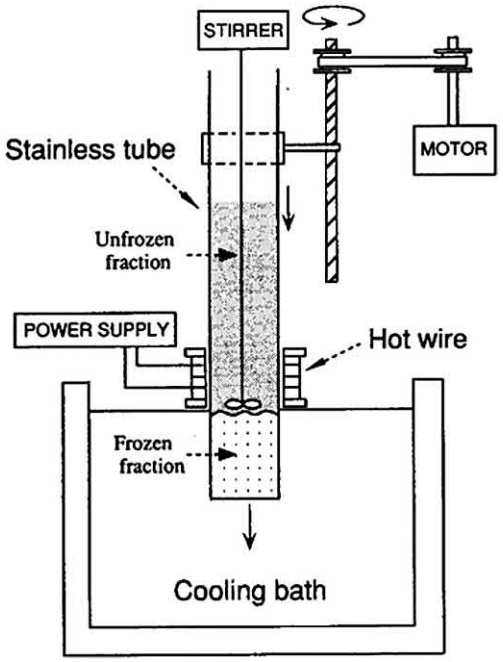

FIGURE 41.6 Apparatus for the progressive Freezing–Melting Process. (From Liu et al. [37])

in the liquid on the efficiency of separation and concentration of glucose and sodium chloride under various freezing rates. They found that efficiency was greatly improved by supersonic radiation and increasing dissolved air concentration. In the progressive freeze-concentration, the major part of the impurity in the ice phase occurred when supercooling occurred before the initial crystallization at the bottom of the sample vessel. Different mechanisms of solute rejection at the interface were observed [99]. Ice-nucleating protein (types of anti-freeze proteins) could be used to suppress initial supercooling [37, 100]. Curran [88] studied the effects of container geometry on the recovery of product water from indirectly frozen saltwater. Saltwater was frozen in containers with a circular or rectangular cross-section, then allowed to melt and drain until the residual ice was potable. Thin rectangular cross-sections were found to be more effective than circular cross-sections. The product water recovery was found to increase with increasing ice height up to 60 cm, beyond which the effect of height was negligible.

The growth rates of ice depend on the ionic salts, surfactants, and water-soluble polymers. Michaels et al. [101] identified that both growth-rate enhancements (up to a factor of about five) and retardations (up to a factor of about three) could be possible in the presence of additives. The type of effect and its magnitude were dependent on the nature of the additive, its concentration, and upon the degree of super-cooling. The effect of solutes may have a consequence of dislocations introduced in the crystal during growth. Miyawaki et al. [102] showed that a tubular ice system with a large cooling surface area was effective as a method for scale-up of progressive FM with an increased productivity and high yield. In this method, the slower growth rate of ice and the higher circulation rate gave the lower effective partition constant as was expected theoretically by the concentration polarization model. The effective partitioning constant was also dependent on the initial solute concentration; the higher concentration gave a higher partitioning constant of solute. By the tubular ice system, coffee extract, tomato juice, and sucrose solution were very effectively concentrated to high concentrations with good yields, showing the high potential of progressive FM for practical applications. The progressive FM process can be easily run at atmospheric pressure and with less complexity. It gives only one block of ice; thus, the separation and melting process is easy.

The main disadvantage is that it requires greater processing times and large temperature differentials [88]. In this case, the partial melting of ice was effective. In this method, the ice crystal formed after progressive FM was melted gradually with time to collect the melted fractions with intervals. Then the initial fractions were found to contain a higher amount of solute. Therefore, the yield could be improved to a necessary level (>90%) by recovering these fractions with the higher solute concentration. The recovered melted ice fraction may be mixed with the feed in the succeeding batch of PFC operation to avoid dilution of the concentrate. The partial ice melting could extend the practical applicability of PFC substantially.

In progressive FH, yield decreases with an increase in solute concentration because of solute inclusion in the ice. Progressive FM was applied for the concentration of sucrose solutions with various concentrations (0.3–40%), a model pear flavor solution, tomato juice (5.3°Brix), and apple juice (12–15°Brix) [103]. In the case of the model solution with pear flavor, the apparent partition coefficient between the ice and the concentrated liquid phase was low to give a high yield, while samples with high solute concentrations showed an increase with an increase in solute concentration to reduce the yield [103]. A partial ice-melting technique overcame the major drawback of progressive FM [103, 104]. Ice cubes from 10% (w/w) sucrose were partially melted, and it was observed that at the lower temperature with relatively faster stirring speed, the initially melted fractions effectively contained a higher concentration of solute than the later-melted fractions. Apple juice with 13.7°Brix was concentrated up to 25.5°Brix by progressive FM using a tubular ice system. Part of the ice was broken down into pieces and used for partial melting by the partial ice-melting vessel. This improved the yield from 63.8 to 85% by recovering the initial 30% melted fraction. In the case of pear juice, after 2.36 times concentration of flavor component (butyl acetate), the yield was improved from 86.7 to 95% by recovering the initial 35% melted fraction. The recovered fraction was recommended to mix with the original solution [103, 104]. A partial ice-melting method was also used for solutions (i.e. fructose, glucose, sucrose, and a simulated juice, 5–20°Brix) to increase overall system efficiency [105]. The ice produced in the four steps of the process retains solutes at levels of 1.0–8.8°Brix (i.e. solute mass fraction in the ice). The recovery of these solutes during thawing can increase overall system efficiency by recovering most of the solutes in the ice. Ten fractions of melted ice at 20°C were

produced. The first thawed fractions showed solute concentrations 1.9–3.3 times higher than the mean solute mass fraction in the ice, while the last fractions were shown to be less than 0.2 times.

The inclusion of solutes is dependent on the initial temperature, on the growth rate, and on the solution concentration. A sweating step after progressive ice crystallization was able to purify ice by operating in-depth draining out of the trapped solution pockets [106–108]. The solution was placed into a tank and the crystallization took place on the external surface of a cooled tube, and the solution is agitated by air bubbling [106, 107]. A fast crystallization step (i.e. 5–14 h) gave quite impure layers, and the use of severe sweating conditions (0°C, 3 h) can lead to salinities lower than 0.5 g/kg, satisfying the drinking water standards. Sweating efficiency depended on temperature gradient, and the main kinetic parameters influencing sweating of ice were initial concentration of ice, sweating temperature, and sweating time [106]. The system of Rich et al. [107] with a seating step included four steps: (i) crystallization of the ice layer by controlling the cooling rate in the tube, (ii) draining off the concentrated brine (iii) purification of the layer by sweating, and (iv) melting of the ice to recover the fresh water. The purity of the crystalline layer was mainly affected by the initial salinity of the brine, and cooling rate. Sweating was able to purify the interior of the ice layer and to reach the drinking water standards.

41.4.1.4 Falling-Film Type

In this type, the solution flows down over the wall of the heat exchanger (well-mixed). Crystals are formed on the wall surface under the falling film. Shear caused by solution flow at the crystal–solution interface increases the mass transfer coefficient and promotes the transport of impurities from the interface to bulk. This process is easy to scale up because of the modular design. Currently, most layer growth processes are used in the chemical industry, and they are seldom used in the food industry.

Local equilibrium at the interface exists, and solute trapping occurs based on the rate of crystal growth [109]. Solutes are distributed between brine and ice crystals. Adsorption on the ice crystal surface and entrapment within the ice may be the major reasons for the presence of the solutes in ice. The distribution or coefficient of salt (ratio of salt in brine and ice) is used to determine the solids in the ice and slurry [110]. Ice growth rates and impurities in ice during layer crystallization of laminar falling film ($50 < Re < 600$) showed that one step layer crystallization had lower separation as compared to the suspension crystallizers with wash columns [111]. In the case of fructose and orange juice, the ice layer on the stainless-steel surface was more pure at low concentrations of soluble solids, low growth rates of ice, high velocity of solution on the plate, and high molecular weight of solutes (i.e. higher molecular weight is less likely to be trapped) [112, 113]. Similarly, low initial concentrations of sucrose, glucose, and fructose produced more purity, and a higher degree of concentration was attained in sucrose solutions in a shorter time as compared to glucose and fructose [114, 115]. In addition, the crystals' orientation is another important factor to eliminate impurities in ice [11, 116].

Falling film FM was used to concentrate oligosaccharides, proteins, and isoflavones from waste-water of tofu processing for developing functional ingredients [117]. The concentration could be increased by a factor of 8 (i.e. 1.9°Brix to a final concentration of 15.5°Brix). A pilot four-stage FM was used to increase the soluble solids content of the whey to 21.8°Brix, and the purity of the ice formed was determined [118]. Falling film FM was used for the concentration of grape juice from 16.4 to 29.5°Brix (Hernandez et al. 2010). It showed an average rate of 1.38°Brix/h with flow rates of 0.8 L/s, and ice production ranged from 1.32 to 1.05 g/m^2 s. In the case of orange juice it showed a linear increase of concentration at a rate of 0.75°Brix/h until a final concentration of 28.8°Brix, and soluts concentration in the ice showed an exponential increase [13].

The drawbacks of a dynamic growth system are that it needs a large solution circulation rate, it needs a large crystallizer volume, a multistage operation is required to attain high purity (sometimes eight to nine stages may be needed), it needs optimization of some variables such as number of stages, reflux ratio, and maximum thickness of crystal layer, the cost and energy efficiency are adversely affected by the need for repeated operation of a batch procedure. A continuous commercial dynamic layer growth process was developed using a counter-current layer crystallization technique called the Bremband belt crystallizer [119]. The main problem of this process is that it is uneconomical.

41.4.1.5 Circular-Tube Type

In this process, ice is formed from a solution flowing through a tube cooled from outside [120–123]. Super-cooling can be obtained in a liquid before it solidifies when forced to flow inside circular tubes. The maximum super-cooling depends on the local tube wall temperature, tube inside diameter, Reynolds number, and a dimensional constant depending on the process [124]. The salt entrainment in the ice layer is one of the major problems in an indirect FM process. Janzow and Chao [125] studied the salt entrainment in ice crystallized from brine on a flat pellet. The ice layer thickness first increases rapidly, reaches a maximum, then decreases. The phenomenon corresponds to the growth and subsequent melting of dendrites. It was believed that brine adhering to the thin plates of ice and perhaps being retained in the interstices by capillary forces is responsible for the relatively high salt concentration found in the melted ice. A rinsing operation could be applied to reduce brine content in the ice layer.

41.4.1.6 Suspension Crystallization Unit

In a suspension crystallization unit, the product to be concentrated is agitated in a vessel cooled by heat transfer through the walls of the jacketed vessel. This vessel may be either a scraped-surface heat exchanger or simply a jacketed kettle vessel. The result of this process is a pumpable suspension of ice crystals in the concentrated product, which must then go to a separation device. Independent control of ice nucleation and crystal growth is very difficult in this type of crystallizer

[5]. Many variations have been investigated to develop a process that allows independent control of ice nucleation and crystal growth [59]. Heterogeneous dendrite ice grows on the wall toward bulk solution due to the large temperature gradient [126]. The induction time of ice fouling was correlated with the degree of supercooling on the cooling wall, and this can be used to estimate the critical time interval between two scraping actions [127]. Ultrasonic radiation was also used for ice nucleation due to the requirement of low supercooling (0.4–0.5°C) [128, 129]. The advantages are (i) an inexpensive plain heat exchanger can be used, (ii) the higher coolant temperature saves capital and operating costs of refrigeration, and (iii) better quality of crystals [130].

A modification of the process above involves the recycling of all or part of the mass in the main vessel. One process recycles the entire crystal slurry through an external heat exchanger, similar to a forced-circulation evaporator, to provide cooling. Nucleation occurs mainly in the heat exchanger, usually scraped surface, while most of the growth occurs in the main vessel. In a slight variation of this system, only the liquid product from the main vessel is recycled by withdrawing the liquid through a filter. This ice-free liquid is then pumped through a scraped-surface heat exchanger operating at a temperature so that liquid remained at high degree supercooled to promote the nucleation of small crystals (50 μm). These fines are then pumped back into the main vessel, where a ripening process occurs. The difference in equilibrium conditions between the fine nuclei and the existing seeds provides the driving force for ripening, which results in the dissolution of the fines and the growth of the seeds as the equilibration process occurs. Large mono-disperse crystals may be formed in this way [5]. In the ripening tank, the unstable sub-critical ice crystals melt and the latent heat they absorb in melting causes freezing on the surface of the large ice crystals in the ripening tank [131]. As small crystals have a lower melting temperature in solution than larger crystals, the small crystals melt and recrystallize on the surface of the larger ones.

The main features of the suspension growth option combined with wash column technology are large single-stage efficiency and large net production per volume of equipment and time period. The suspension option also offers a continuous operation, which may result in lower energy consumption.

A comparison of layer and suspension crystallization growth is given in Table 41.4. Both layer and suspension processes are governed by the same crystallization laws. This means that the results obtained with both process types will, in principle, be the same when the operating conditions are identical. In practice, the main advantages of layer growth in comparison with suspension growth come from simple technology with simple design and scale-up. A high growth rate can be achieved in layer growth processes because heat is transferred through the solid layer. However, a large growth rate usually results in a limited effective distribution coefficient in a single stage. By adding a sweating step and by repeating the operation, high purity can be attained but at higher costs.

Fluidized bed heat exchangers are also used due to their low cost as compared to scrapped-surface heat exchangers [132]. FM of apple juice in a fluidized-bed heat exchanger

TABLE 41.4
Comparison between Layer and Suspension Crystallization Growth

Feature	Layer Growth	Suspension Growth
Purity attainable	Limited per stage (repetition needed)	High in one single stage
Crystal–solution interface	Small (10~100 m^2/m^3)	Large (10,000 m^2/m^3)
Growth rate	Large (10^{-5} ~10^{-7} m/s)	Small (10^{-7} ~10^{-8} m/s)
Purification	Solid–liquid separation, sweating and reprocessing in same unit	Solid–liquid separation, sweating and reprocessing in separate devices
Production rate/volume of equipment	Small	Large
Type of operation	Batch, possibly continuous	Usually continuous
Technical feasibility	Proven technology	Force transport columns: demonstration phase
Heat transfer coefficient	Small (50~400 W/m^2K)	Large (1000~4000 W/m^2K)
Design	Simple	More complicated
Scaling up	Simple	More complicated
Crystal size (distribution)	Not important	Important
Solid–liquid separation	Simple by draining followed by melting	Extra device needed
Pieces of equipment	One single	Several
Moving parts	Only pumps	More moving parts needed
Transport problems	No suspension handling	Pumping of suspension
Incrustation	No, layer is product	Yes, may be hampering
Flexibility	Multipurpose	More tailor-made
Reliability	Restart without loss of product	Loss of product in case of interruption which has to be recycled

Source: Chen [162].

showed that operation remained stable as long as the erosion rate caused by fluidized particles is equal to or greater than the ice growth rate [133].

41.4.2 Externally Cooled

Externally cooled crystallizers employ a heat transfer device external to the main crystallization vessel or channel. One type of externally cooled crystallizing unit employs a heat exchanger to super-cool the liquid feed so that the cold feed provides the cooling effect in the main vessel.

41.4.2.1 Vessel Type (Atmospheric)

Nucleation and subsequent crystal growth both occur in the main vessel. Conditions in the heat exchanger must be closely controlled to prevent nucleation from occurring where it is not wanted [5]. A modification of the external cooling process involves recycling all or part of the mass in the crystallizer. One process recycles the entire crystal slurry through an external heat exchanger to provide cooling. Nucleation occurs mainly in the heat exchanger, usually a scraped surface heat exchanger, while most of the growth occurs in the crystallizer. Ideally, ice-free liquid is cooled to promote nucleation and generate small crystals by being pumped through a scraped surface heat exchanger (SSHE) operating at high supercooling. These fine crystals are then transported with the product into the crystallizer, where a ripening process occurs.

A solution is super-cooled in a heat exchanger without ice formation. Ice crystals are formed instantly in a nucleation device before transport to a separate vessel for growth. The primary aim is to avoid heterogeneous crystallization within the crystallizing unit. The inside wall of the heat exchanger has to be highly polished or coated with a hydrophobic plastic to minimize changes of minimum nucleation and crystallization within the heat exchanger. The concentrated solution was filtered to effect ice separation. Low energy consumption is claimed [134]. Janzow and Chao [135] identified that within a relatively narrow range of super-cooled brine temperatures, large plate-like free ice crystals of up to several inches in length were formed in the bulk of slowly traversing brine simultaneously with the growth of dendrite ice on a cold surface.

It has been reported that ice crystals with 1 mm diameter could be produced by applying the process of ripening vessel [24, 136] with long residence time of ice crystals. Smith and Schwartzberg [137] examined ice crystal size change during ripening. The method of producing large ice crystals, which uses the Ostwald ripening effect, was developed and is now widely used in industry. Shirai et al. [66] and Kobayashi et al. [138] proposed another strategy to make large ice crystals by agglomerating the small ice crystals produced. Theoretical analysis of certain FM systems was also carried out by Bayindirli et al. [110], and Ratkje and Flesland [139]. The agglomeration of ice crystals mainly depended on the seed crystals, initial crystal size distribution, and solute concentration [138]. An extensive agglomeration of ice crystals was observed in low-concentration glucose solution (i.e. 10%, w/w), but not at high concentrations (i.e. 20 and 30%, w/w). However, the Ostwald ripening mechanism in ice suspensions was the most important for crystal size [140]. In order to achieve the optimal function of equipment, the maximum amount of ice in the re-crystallizer must not exceed 40% and the average speed of ice growth was lower in strawberry juice as compared to sugar solutions [141].

41.4.2.2 Vessel Type (Pressure Shift)

Pressure-shift nucleation showed potential advantages over the conventional atmospheric crystallization step at the scraped-surface heat exchanger [142]. Orange juice of several concentrations was crystallized at different pressures and temperatures [142]. It was observed that the higher the final concentration in the juice the smaller the ice crystals formed. The advantages of pressure-shift nucleation over conventional crystallization were (i) quasi-instantaneous formation of ice after expansion, (ii) ice was homogeneously distributed throughout the juice, and (iii) ice formed was round in shape without pockets.

41.4.2.3 Spiral-Finned Type

The spiral-finned crystallizer for progressive freeze concentration is a potentially effective system to concentrate a solution and to produce pure ice crystals. It was designed focusing on increasing the surface area, which affected the rate of heat transfer. A layer of ice crystal was generated on the cooling surface of the crystallizer and adhered to the surface as it grew while coolant was circulated in the cooling jacket to remove heat. This system can offer an attractive alternative for the food industry since it involves no heating and has the ability to produce highly concentrated solution [143]. The efficiency of the system was significantly improved by the lower effective partition constant (i.e. 0.35) and higher solute recovery (i.e. solute recovery of approximately 0.96 g of glucose obtained per 1 g of initial glucose). A maximal ice production of 1.71 g/m^2 s was attained [143].

41.5 VACUUM FREEZING

Vacuum-freezing and vapor-compression systems have been used for seawater desalination. Water can itself serve as the refrigerant in vacuum freezing [144]. In this option, a high vacuum is used to vaporize a portion of water, which then provides the refrigeration effect for lowering the temperature of the product and causing ice crystallization to occur. The washed ice is melted by direct-contact condensation of the water vapor in the melting-condensing unit. All vacuum freezing processes contain a crystallizing unit which is a vessel in which ice crystals and vapor are formed simultaneously by maintaining the vessel close to the triple point (when material could not be considered either solid, liquid, or gaseous) (0.61 kPa). Based on the method by which the vapors are removed, these may be further classified as (i) vacuum-freeze and vapor-compression systems, (ii) absorption-freeze and vapor-compression systems, and (iii) vacuum-freeze and ejector-absorption systems.

In the *vacuum-freeze and vapor-compression method*, a mechanical compressor is used to remove the vapor phase. The vapor is compressed so as to permit it to condense either directly as pure crystals or on a heat-transfer surface. In the case of *absorption-freeze and vapor-compression*, water vapor is absorbed in a material that has a vapor pressure below the triple point and the absorbent has to be regenerated. A conventional refrigeration cycle can be used to provide the heat necessary to drive off the absorbed vapor. The *vacuum-freeze and ejector-absorption* method uses mechanisms to remove the vapor by an absorption cycle or low-pressure steam ejector. The steam that drives the ejector is also used to regenerate the absorbent. The ejector acts as a thermal compressor to raise the quality of the removed vapor so that the vapor can be condensed [23].

In vacuum freezing the compressor must handle a very large volume of low-density water vapor due to the very low vapor pressure of water. Whereas when a relatively volatile refrigerant, such as butane, is used, the freezer pressure is raised to approximately atmospheric pressure, and the volume of vapor to be compressed is greatly reduced. Furthermore, compressor technology for butane from 0.85×10^5 to 1.05×10^5 Pa is much better developed than that for water vapor compression from around 400 Pa. On the other hand, in vacuum freezing, no butane is added to the system, and therefore the complexity and expense of butane recovery, butane make-up, and fire and explosion protection measures are avoided. Hence the relative simplicity of vacuum flash-freezing recommends its use, especially for plants of small size [1]. In the case of vacuum freezing, the residence time is not an important variable, and the process is more heat-transfer controlled, requiring agitation. Moreover, it needs a more efficient design of the melting-unit for the removal of non-condensable gas in the system [26]. Augusto et al. [145] reported the development and application of a mathematical model for the prediction of the low-pressure-vaporization (LPV) process of free water. They focused on defining clearly the two stages of the LPV process (before and after the so-called flash point). The physical domain was divided into two control volumes: (i) the first one contained the mass of free water (i.e. ideally assumed at a uniform temperature); and (ii) the second one included the volume of the vaporization chamber above the water-free surface of the condenser and the vacuum pump. Vacuum crystallization requires semi-continuous operation, limited cost reduction potential, and potential loss of aromas and volatiles from liquid foods [130].

41.5.1 Vapor Compression System

In the vacuum freezing vapor compression process, a large multi-blade compressor is used to compress the vapor from the freezer to the melting-unit. For a plant having a capacity of 227 m^3/day, the compressor is more than 3 m in diameter and needs a fairly high moment of inertia to start. For larger desalting plants of perhaps 4000 m^3/day and above, it is difficult to find a practical compressor [146]. There are two components of a VF system: the vapor removal unit to keep the slurry at or below its triple point and a freezing/evaporation unit to keep ice particles suspended with a fluid slurry–vapor interface. An economical system will have freezer and vapor removal units of nearly the same capacity; both are expensive. In principle, a balance between the size of the evaporation and condensation units could be calculated from existing correlations when standard thermodynamics are known, which is difficult. The primary compressor must compress the water vapor from a pressure somewhat below the vapor pressure of water in equilibrium with the brine at its freezing point up to a pressure somewhat above the vapor pressure of pure water at 0°C. An auxiliary refrigeration cycle is needed to remove the excess energy from the system, for example, standard ammonia cycle removing the heat of condensation of the excess water vapor and rejecting heat to ambient cooling water. Pachter and Barak [147] identified the following modules for increasing the compressor's efficiency: multi-compressor modules, direct-contact and evaporative feed pre-coolers, and multi-stage heat removal compressors of flexible blade type. Burton and Lloyd [148] examined the design considerations of the primary and secondary compressors. Additionally, two other aspects, safety and environment, affecting component specifications were discussed.

41.5.2 Vapor Absorption

In order to realize the low energy consumption advantage of FM, it is necessary to seek a different solution, for example, vacuum freezing ejector absorption process (VFEA) [62, 149, 150]. In an absorptive vacuum freezing process, water evaporates from the freezing solution and condenses on a cold salt solution. Condensing on a flowing cold sodium chloride solution was found to be an inexpensive water vapor removal method. In this process, water vapor is compressed by a combination of steam ejector and absorber loop with the primary energy source being thermal rather than mechanical. The process incorporates an absorption loop, which raises the pressure and temperature of a portion of the vapor evolved in the freezer to a level sufficient to drive the steam ejector. The primary and secondary steam discharged from the ejector is at the stoichiometric temperature required to melt the ice. The absorption loop, in raising the freezer vapor from a level slightly more than 400 Pa to that of the primary steam, acts as a compressor. The ejector has the advantage of no moving parts and is capable of being designed for larger plants. The real novelty was in the fact that the water vapor generated in the freezer is recycled through the system to act as the primary steam for the ejector. The addition of heat was completely separated from the process cycle. For a medium to absorb water vapor, a solution of sodium hydroxide is usually chosen as being most appropriate to the pressures and temperatures in the VFEA process. It is also an inexpensive chemical in comparison to alternate choices and is readily available in large quantities. The average vapor pressure in the absorber is maintained below the crystallizing unit pressure to provide the driving force for vapor absorption. The dilute sodium hydroxide solution from the absorber is heated by the

concentrated solution in the absorber heat exchanger before it enters the concentrator. The main feature of the VFEA process is that it can use low-grade thermal energy, which may be generated by flat plate solar collectors. This combination would seem to be attractive in remote coastal or inland communities [146].

The low operating pressures, below 611 Pa, make the effectiveness of this method dependent on keeping the fraction of non-condensable gas in the vapor phase. Air introduced with the feed and from equipment leaks will accumulate near the condensing interface unless removed by a mechanical pump downstream from the condenser. Non-condensable gas entering the vapor space between the freezing slurry and absorbent will be pushed to the absorbent surface by the water vapor flow impeding water vapor condensation [3, 144]. In many cases, feed saline water intake is first pumped to a vacuum de-aerator in order to remove air and other gases before being pumped into the crystallizing unit [146].

The advantage, which offsets the cost of the low pressure compared to the more usual indirect FM, is that the freezing slurry is chilled uniformly, without a stationary interface between the coolant and the freezing slurry. Stationary interfaces require constant, costly removal of ice or special interface treatment to keep the ice from reducing heat transfer to the coolant. Cost-effective absorber design often is sufficiently complicated that published models and theories are of limited use in predicting performance [151].

41.5.3 High-Pressure Freezing–Melting

The high-pressure freezing during the nucleation stage is effective for very fine control of ice structure size [152]. The advantages are (i) a large amount of the water in the product is frozen instantaneously (i.e. immediately after expansion) and uniformly throughout the volume as compared to the nucleation only on the cold surface or in contact with refrigerant in the traditional systems [153–155], (ii) additional inactivation effects on microbial and/or enzyme due to expansion of solution [130].

The vacuum-freezing and high-pressure ice-melting process was introduced by Cheng and Cheng [156], and improvements were proposed by Cheng et al. [157]. In this process, high-pressure ice-melting (washed ice) and de-sublimation of the low-pressure water vapor are conducted simultaneously and respectively inside and outside of heat-conductive conduits. Thus, fresh water is formed inside of the conduits and a de-sublimated (ice) deposit is formed on the outside of the conduits, which is then melted. In some cases, an in-situ de-sublimate dissolution operation is used. In this case, an aqueous solution is brought into contact with the de-sublimated mass and the conduits are depressurized. The de-sublimate dissolves in the aqueous solution, and an equivalent amount of ice is formed inside of the conduits. It is noted that the result of the in-situ de-sublimate dissolution operation, de-sublimate, is not recovered as product water. The advantages are: it does not require a compressor or absorption solution for the low-pressure vapor, it does not require a regeneration loop or a heat pump (a refrigeration loop) to reuse the heat released in the condensation or de-sublimation of low-pressure water vapor in supplying the latent heat of melting ice, and the process uses commercially available components and can be operated reliably [158]. An extended surface freezer, such as the rotating tray freezer used in this process, is found to have several advantages over the conventional spray freezer or agitated jet evaporator. These advantages are: lost driving force due to back-mixing of brine is lower, the need for a demister is eliminated, power input in the refrigeration is saved although a portion of power is used by the stirrer, and larger crystals can be made so that the crystal washing operation can be facilitated [158].

41.5.4 Vacuum Freezing Multiple Phase Transformation FM Process

In the case of low pressure below the triple point (less than 610 Pa) sublimation occurs and it is called sub-triple point vapor. When a sub-triple point vapor is cooled at constant pressure, it condenses as solid solvent, as ice, and this operation is called de-sublimation. When a super-triple point vapor is cooled at constant pressure, it condenses as liquid. Cheng et al. [159] proposed a vacuum freezing multiple phase transformation (VFMPT) consisting of vacuum evaporation freezing, sub-triple point vapor de-sublimation, de-sublimate melting, super-triple point vapor generation, crystal-washing, and crystal melting. A preliminary economic analysis shows that VFMPT process can be more competitive than reverse osmosis and distillation/evaporation in the range of solute concentrations represented by sea-water desalination both in terms of equipment and operating cost, and in many applications when the concentration is higher VFMPT can be more suitable for a reverse osmosis process, which requires very high pressure. Advantages identified for the VFMPT process are: high heat transfer rates, low energy input, insensitivity to fouling and corrosion, and the ability to handle highly concentrated as well as acidic and alkaline solutions.

41.6 HYBRID SYSTEM

A combination of reverse osmosis and FM could provide an economical alternative for concentrating liquids. The rejected concentration brine disposal from reverse osmosis plants may cause serious environmental impacts. The high concentration of the RO rejected brine limits the choice of the second-stage desalination unit. The energy efficiency of FM makes it a promising choice since the process is independent of fouling, and corrosion is low due to the operation at low temperatures. The economical and energy comparisons between the combined system and separate RO and direct FM units of 200 m^3/h was reported [160]. The combined system can reduce the energy consumption by about 13% and 17% compared to separate RO and direct FM plants, respectively. The combined system can reduce the rejected brine by over 90% of that of a separate RO plant at the same water production. However, the energy savings could be increased to 25% and improvement in water quality could be increased to 71% as compared to the

conventional RO desalination if a heat pump is used in the FM [161]. The use of an electric field and ultrasound-aided process could be used in the freezing-unit in order to enhance the performance. However, all these additions will make the process more complicated.

41.7 CONCLUSION

The FM process has potential for the food industry for concentrating liquid foods. In this process, first ice is formed from part of the water in solution by either direct contact of the coolant or indirect contact as separated by a metal wall. Finally, ice is separated from solution to form concentrated solution and relatively pure water. The main advantages of this process are its low energy requirement and low-temperature operation, thus giving an economic advantage as well as being mild to the solution. There are huge numbers of different types of FM being progressed although the main issue is still its complexity. The recent development of hybrid FM could have economic and quality advantages in liquid food concentration in the food industry.

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42 Microwave Pasteurization and Sterilization of Foods

Jasim Ahmed and Hosahalli S. Ramaswamy

CONTENTS

42.1 INTRODUCTION

Thermal processing remains the preferred processing technology in the food processing industry ever since the discovery of the process by Nicholas Appert and its subsequent commercialization. The objective of thermal processing is to extend the shelf life of food products without compromising food safety. Thermal treatments, including pasteurization and sterilization, can be employed according to the severity of the heat treatment and the intended purpose [1]. Apart from inactivation of pathogens, thermal treatment can also impart some other desirable changes, such as protein coagulation, texture softening, and formation of aromatic components. However, the process has also got some limitations due to the partial destruction of quality attributes of food products, especially heat-labile nutrients and sensory attributes. The technological revolution, nutritional awareness, and continuous demand for the new generation have necessitated the search for new or improved food-processing technologies. Recently, various emerging food processing technologies, including microwave and radiofrequency heating, pulse-electric field treatment, high-pressure processing, ultrasonic applications, irradiation, oscillating magnetic fields, etc., are being investigated to improve, replace, or complement the conventional processing technology.

Microwave heating (MW) of foods is attractive due to its volumetric origin, the rapid increase in temperature, controllable heat deposition, and the easy cleanup opportunities. It is currently being used for a variety of domestic and industrial food preparation and processing applications. It has been used successfully to finish drying of potato chips, precooking of chicken and bacon, proofing and frying doughnuts, tempering of frozen foods, and drying pasta products. MW processing for fresh filled pasta has become common in Italy since the 1990s, and the technology has been applied to ready-to-eat meals, pasta-based products, and a variety of other foods throughout Europe, Japan, and North America [2]. Recently, MW has been used to heat foods in commercial pasteurization and sterilization applications to enhance microbial destruction and promote better product quality. Several European and Japanese food-processing companies have utilized the technology for commercial pasteurization and sterilization of in-packaged foods while North American companies have still not adopted the technology for commercial applications [3].

Microwave heating is preferred for pasteurization and sterilization over conventional heating for the basic reason that the process is rapid and requires the shortest come-up time to the desired process temperature. To process liquid foods, high-temperature-short-time (HTST) processes have been accepted by the food-processing industry to reduce the adverse thermal degradation in food quality while ensuring food safety [4]. However, the HTST process is not suitable for solid foods processed by conventional methods due to slow heat conduction which often causes overheating at the solid surface during the time needed for the heat to be transferred to the slowest heating point of the food [4, 5]. MW heating has the advantage of overcoming the limitation imposed by the slow thermal diffusion process of conventional heating [5]. The volumetric heat generated by MWs can significantly reduce the total heating time and severity at the elevated temperatures needed for commercial sterilization [6] whereby bacterial destruction is enhanced, but thermal degradation of the desired components is reduced.

Study of the destruction kinetics of microorganisms by MW heating has attracted considerable interest since the 1940s when the first work by Fleming [7] was reported, and the argument between non-thermal and thermal effects was born. Several theories have been proposed to explain how electromagnetic fields might kill microorganisms without heat as summarized in a review by Knorr [8]. On the other hand, some researchers [9] refute any molecular effects of electric fields compared with thermal energy using classical axioms of physics and chemistry. Palaniappan and Sastry [10] advocated that the effects of MW and dielectric heating are the fields where there is a knowledge gap, and further studies are required.

42.2 PRINCIPLES OF MICROWAVE HEATING

Microwaves are electromagnetic radio waves that are within a frequency band from 300 MHz to 300 GHz. Microwave heating refers to dielectric heating due to polarization effects at a selected frequency band in a non-conductor. It differs from capacitive heating by the placement of the sample. In capacitive heating, the sample is placed between the electrodes, while the food material is commonly housed inside a closed cavity in the MW heating. Microwave heating applications have been limited to a few narrow frequency bands (Table 42.1) for industrial, scientific, and medical use to avoid interference with the radio frequencies used for telecommunication purposes. The typical bands are 915 ± 25 MHz and 2450 ± 50 MHz with penetration depths ranging from 8 to 22 cm at 915 MHz and 3–8 cm at 2450 MHz depending on moisture content [6]. The latter, in particular, is used more often in domestic MW ovens while both frequencies are used for industrial purposes. It is worthwhile to note that outside of

TABLE 42.1
Frequencies Assigned by the Federal Communications Commission for Industrial, Scientific, and Medical Use

Frequency
13.56 ± 6.68 kHz
27.12 ± 160 kHz
40.68 ± 20kHz
915 ± 25 MHz
2450 ± 50 MHz
5800 ± 75 MHz
24,125 ± 125 MHz

the United States, frequencies of 433.92, 896, and 2375 MHz are also used.

MW heating in foods occurs due to the coupling of electrical energy from an electromagnetic field in a microwave cavity with the food and its subsequent dissipation within the food product. This results in a sharp increase in temperature within the product. MW energy is delivered at a molecular level, through the molecular interaction with the electromagnetic field, in particular, through molecular friction resulting from dipole rotation of polar solvents and the conductive migration of dissolved ions [11]. The principal mechanisms involved in MW heating are, therefore: dipole rotation and ionic polarization. Water in the food is the primary dipolar component responsible for dielectric heating. In an alternating current electric field, the polarity of the field is varied at the rate of MW frequency and molecules attempt to align themselves with the changing field. Heat is generated rapidly as a result of internal molecular friction. The second major mechanism of heating with MWs is through the polarization of ions as a result of the back-and-forth movement of the ionic molecules trying to align themselves with the oscillating electric field. MW heating is also affected by the state of the constituents, whether they are bound or free, e.g., bound ions have much lower microwave absorptivities [12, 13].

The volumetric heating rate (Q) of the microwave at a particular location is related to the electric field strength by

$$Q = 2\pi f \varepsilon_o \varepsilon'' E^2 \tag{42.1}$$

where f is the frequency of microwaves, E is the strength of the electric field of the wave at that location, ε_0 the permittivity of free space (a physical constant), and ε'' is the dielectric loss factor (a material property called dielectric property) representing the material's ability to absorb the wave.

42.2.1 Microwave Generation

The magnetron is the heart of the MW oven. MWs are generated by a magnetron, which is attached to the applicator controlled by a waveguide. The magnetron consists of the two elements of an electron tube—a cathode and an anode each of which is circular with anode resonant cavities (anywhere from 4 to 80). A magnet (permanent or temporary) is placed around the anode to provide a magnetic field. When the cathode is heated using an electrical filament, it gives off negatively charged electrons, which are attracted by the positively charged anode. The magnetic field around the anode causes the electrons to move in an orbital fashion rather than a straight line as they jump from the cathode to the anode under an electrical pressure of 4000 to 6000 volts. As the electrons approach the anode, they pass by the resonator cavities of the anode, and this causes the electrons to oscillate at a very high frequency (2450 MHz or 915 MHz). The high-frequency oscillations of the electrons in the magnetron are picked up by a small antenna on the top of the magnetron tube. These oscillations are transmitted through a waveguide to a feed box from where they are distributed into the oven cavity.

42.3 ADVANTAGES OF MW HEATING

MW has been successfully used to heat, dry, and sterilize many food products. Compared with the conventional method, MW processing offers the following advantages: (i) MW penetrates inside the food materials, and, therefore, cooking takes place throughout the whole volume of food internally, uniformly, and rapidly. It reduces significantly the processing time and energy, (ii) since the heat transfer is fast, nutrients and vitamins contents, as well as flavor, sensory characteristics, and color of food are well-preserved, (iii) ultra-fast pasteurization or sterilization of pumpable fluids minimizes nutrient, color, and flavor losses, (iv) minimum fouling depositions because of the elimination of the hot heat transfer surfaces since the piping used is MW transparent and remains relatively cooler than the product, (v) high heating efficiency (80% or higher efficiency can be achieved), (vi) perfect geometry for clean-in-place (CIP) system, (vii) suitable for heat-sensitive high-viscous and multiphase fluids, (viii) low cost in system maintenance, (ix) heating is silent and does not generate exhaust gas, (x) flat radial temperature profile for most products, (xi) can be combined with other technologies, such as regenerative heat exchangers and infrared heating, for better process performance

42.4 FACTORS AFFECTING MW HEATING

Some physical, thermal, and electrical properties determine the absorption of MW energy and simultaneous heating behavior of food materials in microwave processing. These properties/factors are briefly discussed in the following sections.

42.4.1 Frequency

For food application, only two frequencies are allocated for MW heating (915 MHz and 2450 MHz), and, therefore, these frequencies are of special interest. The corresponding wavelengths of these frequencies are 0.328 and 0.122 m, respectively. The wavelength has special significance as most interactions of the energy and materials take place in that region and generate instantaneous heat due to molecular friction. Food constituents except moisture, lipids, and ash are relatively inert at prescribed MW frequencies. Also, the frequency (or wavelength) dictates equipment components such as magnetron, wave-guide, and to some extent heating volume.

42.4.2 Dielectric Properties

The electrical properties of materials in the context of MW and radiofrequency heating are known as dielectric properties, which provide a measure of how food materials interact with electromagnetic energy. Biological materials may be viewed as non-ideal capacitors in that they can store and dissipate electrical energy from an electromagnetic field and the properties can be expressed with a complex notation. The complex notation is characterized by dielectric permittivity with a real component, dielectric constant, and an imaginary

component, dielectric loss [13]. The dielectric properties of materials are governed by the following equations:

$$\varepsilon = \varepsilon' - j\varepsilon'' \tag{42.2}$$

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \tag{42.3}$$

Where ε' is the dielectric constant, ε'' is the dielectric loss factor of the material, and j is the complex constant. The dielectric constant is a measure of a material's ability to store electric energy, and the loss factor is a measure of its ability to dissipate the electrical energy in the form of heat. Complex permittivity is a measure of a material's ability to couple electrical energy from an MW power generator (magnetron). The dielectric properties of materials mostly govern the heating behavior of food materials during MW heating. The power dissipated per unit volume in the dielectric field is directly related to loss factor (Equation 42.1); however, it may be also dependent on the dielectric constant subject to geometry and field configuration [14]. The ratio of the dielectric loss to the dielectric constant, defined as the loss tangent (Equation 42.3), is related to the material's susceptibility to being penetrated by an electrical field and to dissipate (attenuate) electrical energy as heat. Materials are classified by loss tangent. Those materials that are highly lossy absorb MW energy efficiently, whereas highly transparent materials have low loss factors, such as Teflon, glass, and kerosene, and absorb less MW energy.

42.4.3 Moisture Content

The moisture content significantly affects the dielectric properties of the food product and consequently, the penetration depth of the MW. The uneven heating rate is observed in high-moisture foods because of low MW penetration depth. Low-moisture foods will have a more uniform heating rate because of the deeper MW penetration [15]. The initial moisture content of the product and the rate of moisture evaporation play important roles during MW heating. The heating behavior of water is also phase dependent (liquid water versus solid ice phase) and also depends on the available free water content. At constant temperature, the dielectric behavior of free water remains constant in the lower frequency range (static region) and water dipoles have enough time to reorient themselves with not much absorption of energy, while a significant decrease in dielectric behavior can be observed at the higher frequency (optical region) with no field reversal by the water diploes. The dielectric constant decreases exponentially with frequency (critical frequency) in between the static and optical regions. Phase change results in a significant change in the dielectric properties, and, therefore, these properties for water and ice largely differ in their magnitude.

42.4.4 Mass

A direct relationship exists between the mass and the amount of absorbed MW power, which should be applied to achieve the desired heating. For a smaller mass, the batch oven is suitable, while a larger throughput would often be better in large-capacity conveyorized equipment. Such equipment has the added advantage of providing greater heating uniformity by moving the product through the MW field. Each MW oven has a critical (minimum) sample mass for its efficient operation. It is usually a load of around 250 mL of water in a 1 kW oven. Below this level, a significant amount of MW power is not absorbed into the product, and at very low loads, they may damage the magnetron.

42.4.5 Temperature

MW heating is significantly affected by the level of sample temperature. Dielectric properties may vary with temperature, depending upon the material. Both temperature and moisture content can change during heating, and, therefore, those may have a combined effect on the dielectric constant, dielectric loss factor, and loss tangent, and subsequently on the heating behavior. Freezing has a major effect on a material's heating ability because of the vastly different dielectric properties of ice and water. Water has significantly higher magnitudes of dielectric constant and loss as compared to ice, and these properties are also dependent on the MW frequency [16].

The initial temperature of the food product being heated by MWs should either be controlled or known, so the MW power can be adjusted to obtain uniform final temperatures. If the MW oven is pre-set to increase the product temperature from 20 to 80°C, it will practically reach a target temperature of 95°C with an initial product temperature of 35°C. To compensate for the effect of higher initial temperature, the power of the MW oven should be reduced, or a higher sample mass should be used, or the product should be heated for a shorter duration.

42.4.6 Geometry and Location of Foods

The shape of the food product does play an important role in the distribution of heat within the product heated in an MW oven. It affects the depth of MW penetration and affects the heating rate and uniformity. Irregular-shaped products are subjected to non-uniform heating due to the difference in product thickness [15]. The closer the size (thickness) is to the wavelength, the higher will be the center temperature. Smaller particulates require less heat than larger ones. Also, the more regular the shape is, the more uniform will be the heat distribution within the product. A food of a spherical or cylindrical shape heats more evenly than a square. A higher surface-to-volume ratio enhances the heating rate. Therefore, the heating rate for a sphere will be different from that for a cylinder with the same volume. The relationship between load geometry, load orientation, and oven cavity parameters such as cavity size and geometry, however, are not fully established. For most foods, size and geometry in combination with the energy of a relatively small wavelength such as 2450 MHz would result in non-homogeneous but predictable heating profiles. Recently, it has been advocated that MW

heating uniformity of multi-component foods is dependent on food component placement and the geometry of products and packages [17]. Placement has the most significant effect. The temperature distribution could be balanced partly by taking advantage of edge and corner heating intensification.

42.4.7 Thermal Properties

The heating characteristics of foods are dependent to a greater or lesser extent on some thermal properties such as thermal conductivity, density, and heat capacity. The thermal conductivity of food plays a significant role in MW heating. Materials with higher thermal conductivity dissipate heat faster than the ones with lower conductivity during MW heating. Food with high thermal conductivity will take less time to attain uniform temperature during the holding period. The thermal conductivity of frozen food is higher due to the high thermal conductivity of ice, while freeze-dried foods have lower thermal conductivity. The heat capacity of food measures the temperature response of food as a result of heat input or removal. Heat capacity can be raised by increasing solid content by adding components like salt and protein. Heat capacity along with thermal conductivity and thermal diffusivity constitute the thermal properties of the material. The combination of heat capacity with thermal conductivity and density is represented by thermal diffusivity, defined as the ratio of thermal conductivity to the product's volumetric heat capacity:

$$\alpha = k / \rho c_p \tag{42.4}$$

42.4.8 Secondary Flow in Curved Pipe

Thermal processing requires the coldest point to experience a target minimum temperature for a specified residence time. The coldest point in a continuous-flow process is the region where fluids exhibit the maximum velocity, which is the central axial position in a straight tube. The maximum velocity can vary in a helical coil; therefore, the flow characteristics should be determined. The use of helical coils creates a secondary flow due to the momentum transfer in the radial direction, which ensures better mixing and stabilizes the laminar flow [18]. Dean number (*De*) quantifies this phenomenon and therefore is the dimensionless parameter to characterize flow in helical coils [18].

$$De = \mathrm{Re}\sqrt{D_{tube} / D}_{coil} \tag{42.5}$$

$$\mathrm{Re} = \frac{VD_{tube}\rho}{\mu} \tag{42.6}$$

where Re is the Reynolds number; ρ is the density of the fluid (kg m^{-3}), μ is the viscosity of the fluid (Pa s), D_{tube} is the inside diameter of tube (m), and D_{coil} is coil diameter (m). The secondary flow enhances heat and mass transfer rates in addition to the rate of momentum transfer, the latter one resulting in an increased pressure drop [18]. The heat transfer rates are found to increase by a few percent to several-fold in a helical coil; however, they are a function of types of flow regime (laminar or turbulent), fluid properties, and helix configuration. Recently, the concept of Dean number has been used in MW heating of fluid foods and a Dean number exceeding 100, normally exhibiting plug flow behavior, has been found to be suitable for heating liquids in an MW oven where fluid particles with maximum velocity span across most of the tube [19].

42.5 INDUSTRIAL APPLICATIONS OF MW HEATING

The major industrial applications of MW heating are tempering of frozen meat and poultry products, precooking of bacon for foodservice, sausage cooking, drying of various foods, baking of bread, biscuits, and confectionery, thawing of frozen products, blanching of vegetables, heating and sterilizing of fast food, cooked meals, and cereals, and pasteurization and sterilization of various foods. Brief accounts of individual applications are given below followed by a detailed account of pasteurization and sterilization applications.

42.5.1 Tempering of Fish, Meat, and Poultry

The largest use of industrial MW processing of food has been the tempering of meat for further processing [3]. MW tempering is the process where the temperature of the product is raised from storage temperature (generally below –18°C) to a temperature just below freezing point. In the meat-processing industry, the meat used is usually obtained in thick frozen blocks below –18°C. The first operation on the frozen meats usually is to dice, slice, or separate individual sections into smaller pieces. The mechanical operation requires that the blocks be tempered from their solid frozen state to a point where cutting or separation can be carried out easily without damage to the product. Conventional tempering techniques either with water or air subject the outer surfaces of the product bulk to warmer temperatures for long enough for the heat to penetrate to the center. This results in large temperature gradients. In addition, the conventional tempering process takes a long time (several days) with considerable drip loss especially resulting in loss of protein which represents an economic loss. MWs can easily penetrate the whole frozen product, thus effectively reaching the inner regions within a short time. The MW tempering can be performed in a few minutes for a large number of frozen products (5–10 min for 20–40 kg). The temperature to which a product must be tempered depends upon the type of cutting, slicing, chopping, etc., and also upon product compositions such as the combinations of water, salts, proteins, and fats.

As MWs are absorbed by the material, their intensity is attenuated by the penetration depth. Surface layers retain more energy and heat up faster compared to the inner regions of the product. The loss factor increases with the temperature, the product surface heats up faster and faster, and the penetration depth simultaneously decreases. The lower frequency (915 MHz band) has an advantage for the tempering of thick products because of its deeper penetration and longer

wavelength compared to the higher frequency (2450 MHz) MW. Presently, most food industries use MW at 915 MHz for tempering purposes except where the law does not permit the use of this frequency. Tempering of frozen foods is carried out either in a batch or continuous type MW system (25–120 kW). Presently, manufacturers design the system as per customers' choice, type of food products, and applications. The process has been successfully used by the meat, fish, and poultry industries for further processing while the dairy industry has exploited the technology to reduce the chances of rancidity during bulk freezing of butter.

42.5.2 Precooking of Bacon

Precooking of bacon is the second-largest application of MW heating in the food industry [3]. MW heating is found to be an ideal system for cooking bacon compared to conventional grilling. It is reported that about half of total bacon usage is in foodservice, and virtually all foodservice bacon is precooked in MW ovens. In addition, about 10% of the bacon sold in the supermarket is MW precooked.

As a two-component food, bacon loses the fat component, and the desirable characteristics/quality, rapidly during grilling. MW heating of bacon produces a better structure with less shrinkage. Bacon cookers have changed with time and demand. Earlier, a combination of MW energy and hot air was used in an MW environment [6]. Hot air was used to trap the moisture evaporated during the cooking of bacon. A combination of steam, hot air, and MW energy is also used to cook the bacon. A sufficient amount of fat along with trapped moisture is removed during the heating of bacon at temperatures in the range of 70–80°C using steam. The trapped moisture does not convert to steam and, therefore, is removed along with fat. A complete MW system has also been used for bacon cooking where series of magnetrons (equal input) are used to heat and cook the bacon. The placement of the magnetrons varies among manufacturers.

42.5.3 Cooking Sausage

The third-largest application of MW processing is sausage cooking [3]. The sausage patty quality could be improved along with better yield by using the MW process. In sausage cooking, MW processing is used to reduce drip loss (i.e., loss of water), fat, nutrients, and flavor. Various laboratory-scale systems have been developed for MW processing of sausage but not with much commercial success.

42.5.4 Baking

The first commercial success of MW/radiofrequency energy was in the baking industry [6]. Baking ovens use radiant energy and operate in an unspecified frequency to dry the surface and make porous crust of bread. The first bread baking (proofing) was reported by Fetty [20] at 2450 MHz. A combination of MW and thermal energy was further used to produce a brown and crusted loaf in a short time. Schiffmann et al. [21] patented a bread baking technique in which a conventional heat source along with the MW energy was used in combination, and it was claimed to reduce the baking time by 50%. The unit operations associated with baking, in particular, proofing and baking, lend themselves to MW processing, which significantly improves the heat transfer during processing. Numerous reports are available on the baking of cakes, doughnut processing, and frying.

42.5.5 Drying

MW heating offers distinct benefits in dehydration because of its penetration depth, and the uniform heating results in water vaporizing from throughout the product. This induces an inner pressure that maintains the puffed character of the dried product and preserves color, flavor, and nutritional value. MW drying is rapid and more energy-efficient compared with conventional hot air drying [6]. In MW drying, the removal of moisture is accelerated, and, furthermore, heat transfer to the solid is slowed down significantly owing to the absence of convection. The usual practice of applying MWs to the drying of food materials is at the falling rate period where the migration of water from the center of the products is significantly reduced and drying rate is comparatively slow in the conventional drying process. A two-stage drying process involving initial forced-air convective drying, followed by MW finish-drying, has been reported to give better product quality with considerable savings in energy and time. The bakery industry conventionally uses MWs to finish drying of biscuits and cookies. Moreover, moist bakery products exhibit a higher loss factor (ε''), resulting in more heat generation compared to drying at a later stage with minimum water content.

Potato chips are a very popular fast food around the world. The first large-scale application of MW energy in the food processing industry was the finish drying of potato chips. Conventional drying technology cannot rapidly achieve the desired low moisture levels of potato chips and, also, browning creates another quality problem for the product due to the presence of sugars. Drying of potato chips up to 6–8% followed by finish drying by MW overcomes the difficulty of the process industry.

The application of MW energy in vacuum results in an increase in product temperature; however, the temperature rise is limited to the boiling point of the water at the lowered pressure. At a pressure of 3 kPa, free water boils at 22°C. This maintains a product temperature at a level below the temperature used under atmospheric conditions. MW vacuum dehydration was first used for the concentration of citrus juice in France [6]. MW vacuum drying of agricultural commodities includes various cereal grains, and further, this technology has been adapted to grapes for the production of Grape Puffs™ using zoned MW vacuum dehydration [22]. Applications of MWs for the drying of fruits and vegetables are numerous in the literature. The drying of pasta products and noodles at 915 MHz is a commercial success in many countries.

42.5.6 Blanching of Vegetables

Blanching, a unit operation mostly used in canning, freezing, and dehydration of vegetables, involves short time exposure of the product to boiling water, steam, or MW for the primary purpose of inactivating the oxidative enzymes which otherwise would cause an undesirable change in color, flavor, and texture of the finished product during storage. In canning, the blanching process serves to reduce the microbial load, eliminate dissolved oxygen from the product, and to facilitate better packing of the product into cans. Additionally, it can improve the color, flavor, and sensory characteristics of the product. Water and steam are the media commonly used for blanching. Still, convectional steam blanching remains the most commonly employed method in the food industry. Although it is relatively energy-intensive, however, it retains minerals and water-soluble vitamins better than the conventional water blanching. First MW blanching was reported by Proctor and Goldblith [23] using 3000 MHz for some green vegetables, and the technique was useful for enzyme inactivation. The process can retain vitamin C at the highest level. Most of the results indicated that MW blanching was more effective in retaining water-soluble vitamins in vegetables compared to conventional blanching methods. The inactivation of polyphenol oxidase (PPO) and peroxidase (POX) in red beet was compared using traditional and MW (MW) blanching, and it was found that POX was the more heat-resistant enzyme [24]. At a reference temperature of 90°C, it was observed that, in general, D_r values for POX were smaller than for PPO for both MW and traditional blanching.

42.5.7 MW Effects on Enzyme

Enzymes are probably the simplest system to consider for studying bioelectromagnetic effects in living systems. The effect of MWs (0.3–300 GHz) on living matter has been widely studied, and most of the observed effects have generally been explained by purely bulk heating, i.e., temperature increase induced by the electromagnetic field, according to the classical theory of lossy dielectrics [13]. On the other hand, evidence of MW effects not only related to temperature, as measurable by ordinary means, has accumulated over recent years, but the mechanisms involved are still largely unknown due to experimental and modeling difficulties. The inactivation of many enzymes like wheat germ lipase, soybean lipoxygenase, and pectin methylesterase (PME) was studied at various temperatures using conventional and MW batch heating and found to have higher enzyme destruction rates under MW heating conditions [25–27]. This difference is believed to be associated with some contributory enhanced thermal effects of MWs for enzyme inactivation.

42.5.8 Puffing and Foaming

Ultra-rapid internal heating by MWs causes puffing or foaming when the rate of heat transfer is made greater than the rate of vapor transferred from the product interior to outside. MWs are ideal for producing puffed snack foods.

42.5.9 Concentration

MW heating has also been used to concentrate heat-sensitive solutions and slurries at relatively low temperatures. The process is also applicable to highly corrosive or viscous solutions.

42.6 RECENT DEVELOPMENT IN MW FOOD AND PACKAGING

Recently, food processors have developed many new-generation microwaveable foods with suitable packaging materials to meet these demands. The foods are microwavable and for use at home as well as away from home, via convenience-store or office MW. In many cases, the products' success hinges on a combination of product reformulation and package redesign. High-density polypropylene (HDPP) is a low-cost solution for the MW process over other materials that can withstand the target temperature. For sterilization, PET, HDPP, and various polyester-based materials are available as high-quality trays, pouches, and bags. Glass also is a possibility. The metal cap in glass jar proved to be an advantage.

Some of the products available on the market include Eggology's On-the-go 100% egg whites and Marks and Spencer's Steam Cuisine [28]. The microwaveable product expands during cooking, and the lid automatically pops up. A Steam Cuisine fresh prepared meal requires 6 minutes to cook from the raw stage. Another claim has been made regarding the development of "intelligent double pressure cooking technology" where the first cooking occurs by MWs passing through the specially developed packaging and heating the frozen food materials. The frozen water becomes steam and cooks the food from inside out. In the next phase, as the steams comes out of the food tissue, it is held and retained inside the packaging. As the steam pressure builds, the food cooks from the outside in.

Packaging has played a significant role in the MW processing of foods. Big names like Kraft have developed many new packaging materials that can be used in MW heating of pizza [28]. Another development in MW foods has been reported by introducing aroma in the packaging material. The aroma releases during MW heating of the packaged material. In the future, we may expect more revolution in MW package design to satisfy the never-ending demands of consumers.

Uniform heating of food materials by microwaves is influenced by the package design and some other factors including the composition of food, geometry, and oven design. MW heating of packaged foods (e.g., ready meals) happens when the pressure builds up within the package. In-pack pasteurization can be achieved by specially designed packaging including trays, films, and valves. The shape of the trays is mostly optimized for uniform heating, and they are sealed with plastic films including a valve. Sometimes, various active packaging is employed for microwave heating, in particular, for pizzas, and pies. To obtain uniform heating of pizza, susceptor packages have been used to achieve enough crispiness after microwave heating. A US patent (6414290 B1) describes how susceptor can be employed for uniform heating and browning

of pizza or ready meals with circular shapes. A microwave susceptor consists of a layer of metalized plastic film laminated to a dimensionally stable substrate, such as paperboard. The thickness of the metal is designed in such a way that the metal can absorb MW energy and converts it into heat. Such susceptors are commonly used commercially to brown and crispen food in contact with the susceptor. The improvement in heating is due to the escape of vapor through the apertures, which allows the pizza to remain in contact with the susceptor. However, providing apertures in the susceptor requires a separate step in the manufacture of the susceptor and produces chad that must be disposed of. It also destroys the integrity of the susceptor, which forms part of the package for the pizza.

42.7 MW PASTEURIZATION AND STERILIZATION

Pasteurization is the process that uses a relatively mild heat treatment on foods to kill key pathogens, and inactivate vegetative bacteria and enzymes to make food safe for consumption. Most frequently, milk and fresh fruit juices are pasteurized where the minimum process is necessary to eliminate associated health hazards. However, the thermal treatment given does not kill bacterial spores, and hence the product is not stable at room temperature. Under refrigerated storage conditions, one can expect 2–6 weeks of shelf life. Recently, the process has been upgraded to remove the potential health hazards due to *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes*. Pasteurization of milk is achieved by 30 minutes heating at 63°C or 15 seconds at 72°C. Much higher temperatures have also been used for a shorter period in HTST and ultra-high-temperature (UHT) processes. The temperatures and times are determined by what is necessary to destroy pathogenic heat-resistant disease-causing microorganisms that may be found in the food. After pasteurization the product is then quickly cooled to 4°C. Pasteurization temperatures and times vary, depending on the product nature and the target organism.

Sterilization is a more severe thermal treatment of foods. Traditionally, the process is designed to achieve commercial sterility of the products giving them longer-term shelf stability. The magnitude of thermal treatment is a function of pH, and it accounts for the effects of pH on the thermal resistance of the microbial spores. Foods with high pH (>4.5) support the growth of *Clostridium botulinum* which produces an exotoxin. It is recognized that a thermal process sufficient to eliminate toxin-producing *C. botulinum* from the food should make the food commercially sterile if adequately packaged under vacuum, preventing recontamination, and appropriately stored at room temperature (at <30°C to prevent the growth of thermophilic bacterial spores). Commonly, saturated steam at elevated pressures (135–140°C) and steam-heated hot water are used as heating media for food sterilization. MW sterilization has been studied for potential commercial applications. However, the commercialization has faced several problems with some limited success [29].

Both pasteurization and sterilization are based on time–temperature combination processes applied to food products to achieve intended target lethality. In most cases, target microorganisms are chosen for specific types of food. The death kinetics of the microorganisms play a major role in selecting the target lethality, and therefore, the quantification and accommodation of microbial destruction kinetics is an important step in establishing the thermal process.

42.8 KINETICS OF MICROBIAL DESTRUCTION

The destruction of microorganisms and inactivation of enzymes is generally expressed by nth order chemical reaction as:

$$\frac{dC}{dt} = -kC^n \tag{42.7}$$

where dC/dt is the change in concentration C or microbial population (change C to N) with time t, k is the reaction rate constant, n is the order of reaction. Generally, the destruction of microorganisms is described by first-order reaction kinetics. The thermal resistance of microorganisms is also conventionally characterized in food processing by means of a decimal reduction time (D value, heating time at a given temperature that achieves 90% destruction of the existing microbial population) and the thermal resistance constant (z value, the temperature range between which the D values change by order of 10) [30].

$$D_{T_{ref}} = 2.303 / k \tag{42.8}$$

$$z = \frac{T_2 - T_1}{\log D_1 / D_2} \tag{42.9}$$

The equivalent time necessary for thermal treatment with the known thermal resistance of a microorganism is calculated by integration of the time–temperature history using Equation 42.10:

$$F = \int_0^t 10^{\frac{T(t)-T_R}{z}} dt \tag{42.10}$$

This approach has been commonly used in the thermal processes calculations. A similar concept can be applied for determining kinetics parameters during MW heating; however, non-isothermal heating conditions are involved in this case. Resulting D values can be computed using Equation 42.11:

$$D = t_{eff} / \log(C_o / C) \tag{42.11}$$

where t_{eff} is effective time (similar to F as in Equation 42.10) with T_R as exit temperature of the product, obtained using either model-predicted or experimentally determined time–temperature profiles; C_o and C are initial and final

concentrations of microbial cells. The use of this approach is rather limited in studies on MW effects. Only a few studies reported in the literature describing kinetics during MW heating make use of the transient temperature profiles [27]. But as in thermal destruction, MW destruction kinetics of food constituents such as quality characteristics, enzymes, and microorganisms are required for establishing MW processing.

42.8.1 Come-up Time and Come-down Profiles Corrections

Continuous-flow MW heating has advantages over batch processing where the presence of non-uniform temperature in the product has seriously limited its use. Continuous-flow liquid systems allow the maintenance of the time and temperature achieved in steady-state, the recording of average temperatures during heating, the minimization of the temperature gradient by mixing of the liquid, and the cooling of the liquid immediately at the exit. MW heating involves a non-isothermal come-up time (CUT) inside the oven or cavity. The holding and come-down phases take place generally outside the MW oven. Evaluating kinetic parameters during non-isothermal continuous-flow heating are described in detail elsewhere [26]. From a regression of residual log numbers of survivors versus residence time (uncorrected heating time), the first estimate of D values at the exit temperatures can be obtained, and using them at different temperatures one can get an estimate of the z value. Using this estimated z value and the time–temperature profile, a more effective heating can be computed from Equation 42.10. These effective times can then be used to recalculate D and z. These two steps are repeated several times for the convergence of z value. The come-down period contribution occurs outside the MW oven, and this should be subtracted from destruction to estimate the destruction due to MW heating. To do this, the effective cooling time (t_c) can be computed using the same Equation 42.10 with the z value obtained from conventional thermal destruction studies. The extent of logarithmic thermal destruction (*LTD*) during cooling can be estimated following the relationship:

$$LTD = t_c / D \tag{42.12}$$

Where *D* is the *D* value at the exit temperature obtained from thermal destruction studies. This calculated value is then subtracted from the combined destruction of the microbial population due to MW heating and cooling. Microbial destruction data of test samples can thus be corrected for both come-up and come-down period contribution to lethality. This approach has been used in some studies for comparative evaluation of microbial lethality under conventional and MW heating.

42.8.2 MW Heating Systems

42.8.2.1 Batch Heating

In the batch process, the magnetron-based MW ovens are commonly used for heating purposes. The food sample is placed in the oven for a predetermined time to achieve a target temperature. The power level is normally adjusted to achieve a certain desired temperature difference in a given time frame. The volumetric heat absorbed by the food material during MW heating can be calculated by using the following relationship assuming there is no surrounding heat loss:

$$Q = mC_p(T_f - T_i) \tag{42.13}$$

where m = mass of food (kg); C_p = specific heat (kJ/kg°C); T_f = final temperature (°C); and T_i = initial temperature (°C). The absorbed MW power (P) can be calculated by volumetric heat divided by heating time. P is compared with the nominal MW output to compute the efficiency, and generally over 90% of the nominal power can be used by a large sample.

42.8.2.2 Continuous-Flow Heating

Recently, particular interest has arisen in MW heating in continuous systems, because of its potential benefits to the food-processing industry. In MW heating, the radiant energy heats the product directly, without heating the tube walls, and therefore, MW can penetrate the food to achieve more effective bulk heating. Continuous systems have advantages over the batch ones with increased productivity, a quick rise in temperature, controllable heat distribution, easier cleanup, and automation. Several laboratory-based continuous-flow MW heating systems have been used for fluid foods with different configurations [19, 26, 31–33]. The schematic diagram of a basic continuous flow MW heating is shown in Figure 42.1. The raw fluid is pumped (peristaltic pump) through Teflon or glass helical coils placed inside one or several MW ovens connected in series for heating (alternately several magnetrons can be arranged in one oven). Outside the MW oven, the fluid then passes through a holding section to allow a predefined holding time followed by chilling in some form of a tubular heat exchanger. Thermocouples are used for gathering samples temperatures at the entry and exit sections (external to the oven) while fiber-optic probes are used to monitor the temperature inside the cavity/oven.

To establish steady-state flow-conditions for MW heating, the fluid food is pre-circulated in the system after which the MW ovens are turned on. The volumetric flow rates are determined by various pump settings. For the pasteurization process, the flow rate is set, so that the required exit temperature of the fluid in the MW oven is maintained in the holding section. The temperature of the fluid in the holding section is maintained by an external means [26], or in some cases, the exit temperature is elevated to a slightly higher level than required to allow for the heat loss through the insulated holding tubes [19]. However, time–temperature profiles of most of the cases are generated by transit time–temperature measurement at selected regions.

42.8.2.3 MW-Assisted Pasteurization System (MAPS)

The MW-assisted pasteurization system (MAPS) is a potential thermal processing technology, which is used for the pasteurization of food. MAPS uses MW power as a heating source to inactivate microbes to produce safe prepackaged

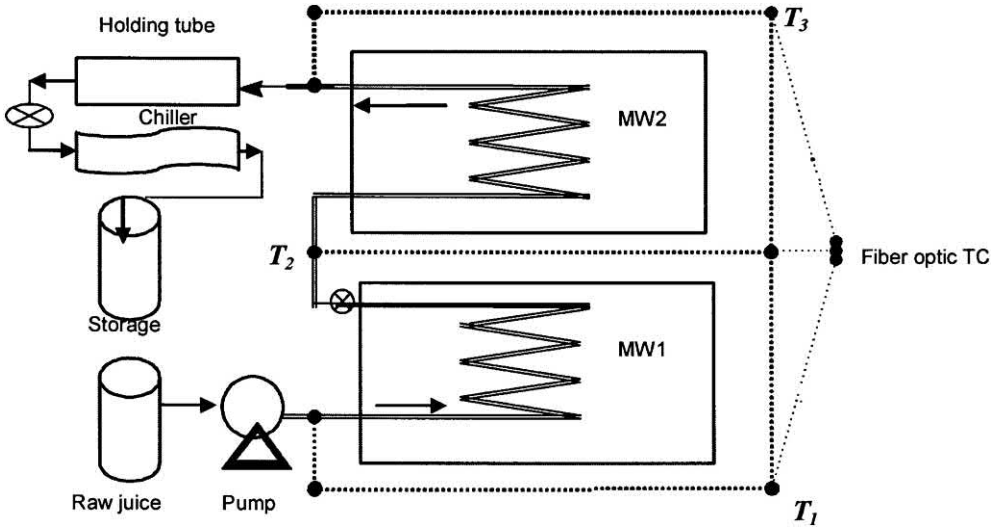

FIGURE 42.1 Continuous-flow microwave pasteurizer.

foods [34, 35]. The current technology differs from an earlier MW-assisted pressurized sterilization system [36, 37]. Recently, researchers from Washington State University (WSU) employed MW heating in combination with hot water immersion in producing higher quality food products, especially ready-to-eat chilled prepared meals. Hong et al. [38] described a 15-kW 915 MHz pilot-scale MAPS for packaged food as illustrated in Figure 42.2. The system is similar to a conventional pasteurization system, which consists of three operating sections, namely, preheating, heating, and cooling. In a continuous process, the products are transported by a conveyor from one section to another. In the preheating section, the packaged food products are loaded and heated in a hot water tunnel to an elevated temperature. In the second section, a pulse of MW energy is introduced to heat the products in combination with hot water immersion and stabilization in a tunnel to raise the internal temperature to a target heating temperature. The pasteurized products are cooled in the cooling section. Water is used for heat transfer, and the residence time in each section is controlled by the speed of the conveyor. It was observed that MW heating improved the heat transfer in the heating section of the MAPS. The difference in the heat transfer values in the packaged products was mostly attributed to the product dimension (mainly thickness) and weight, composition and amount of sauces used, surface area, and dielectric properties of products.

Hong et al. [38] tested MAP for two products, namely, 10 oz. beef meatball in tomato sauce trays and 16 oz. salmon fillet in sauce trays using non-proteolytic *C. botulinum* Type B and *C. botulinum* Type E spores as the target microorganisms for thermal processing. About 57% and 62% of the total lethality for beef meatball and salmon fillet were achieved in the MAH section; the remaining lethality is contributed by the cooling section, whereas the lethality achieved in the preheating section is almost zero The lethality accumulated in the cooling section can be attributed to residual heat and the relatively high temperature in the products as they enter the cooling section. A Monte Carlo simulation indicated that the total lethality of the products follows normal distributions. The simulation showed that more than 98.8% of the process could achieve a minimum of a 5-log reduction of the spores of *C. botulinum* Type B in beef meatball trays against a target lethality of 6-log reductions, and more than 99.1% of the processes achieve >5-log reductions in the spores of *C. botulinum* Type E in salmon fillet trays.

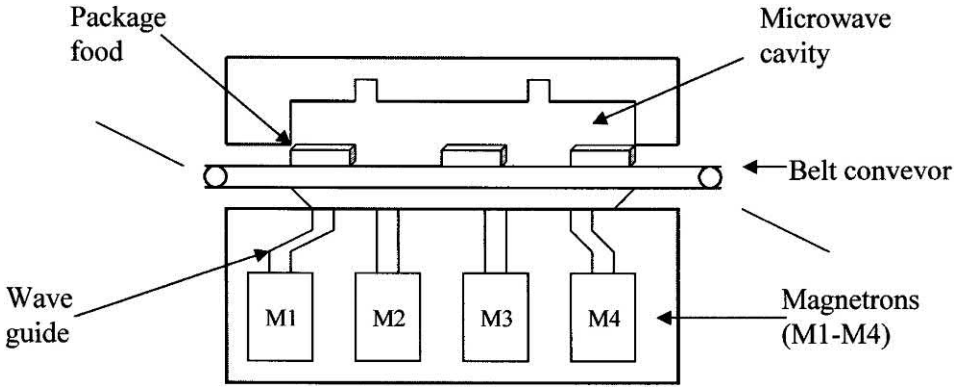

FIGURE 42.2 A typical microwave pasteurizer.

42.8.2.4 MW-Assisted Thermal Sterilization (MATS) System

Researchers from WSU developed a 915 MHz single model MW-assisted thermal sterilization (MATS) system to explore industrial applications of MW volumetric heating in thermal processing of pre-packaged foods [37]. The MATS process of prepackaging mashed potato and salmon fillet has been accepted by the FDA for sterilization of pre-packaged low-acid food in the US.

The pilot MW sterilization system used at WSU is illustrated in Figure 42.3 [37]. The unit consists of two 5-kW 915-MHz MW generators, waveguides, two MW heating cavities, loading and unloading cavities, a sample tray conveyor system, a water circulation system, and a control and data acquisition system. MW power from the generators is transmitted to the MW heating cavities through waveguides during the operation. The food trays are loaded through the loading cavity, which also serves as a pre-heating cavity, and the unloading cavity is used for unloading processed food trays and also works as a cooling cavity. The water circulation system, consisting of two plate heat exchangers, a storage tank, and fixture to provide compressed air to pressurize water, is used to provide temperature-conditioned pressurized water for pre-heating, auxiliary heating to MW treatment, holding, and cooling of food packages. The data acquisition system, data loggers, and custom-built software are used to maintain and collect the operation parameters. The design of the MW applicators in the MW heating section determines heating uniformity and processing time and, thus, is one of the most important parts of a MATS system.

For sterilization, the sample trays were first preloaded on the conveyor mesh belt with a preheating temperature of 60°C with hot water at 122°C in the loading section, and thereafter, the trays were moved through the MW heating cavities and treated by both MW energy and hot water (122°C) with a preset time speed schedule. The sample trays were held in the holding cavity to achieve the desired F_0 after MW treatment. Finally, the trays were cooled to the center temperature of 75°C with tap water and unloaded from the system. *Clostridium sporogenes* (PA 3679, batch No. 307) spores were used in inoculated pack studies for microbial validation of the process. The trays subjected to the target process (F_0 = 6 min) and over-target process (F_0 = 12 min) showed no evidence of gas production during 3 months of incubation. Therefore, the method used in this study was feasible for developing MW processing of inhomogeneous foods, and the developed processing was effective for the sterilization of sliced beef in pre-packaged gravy trays.

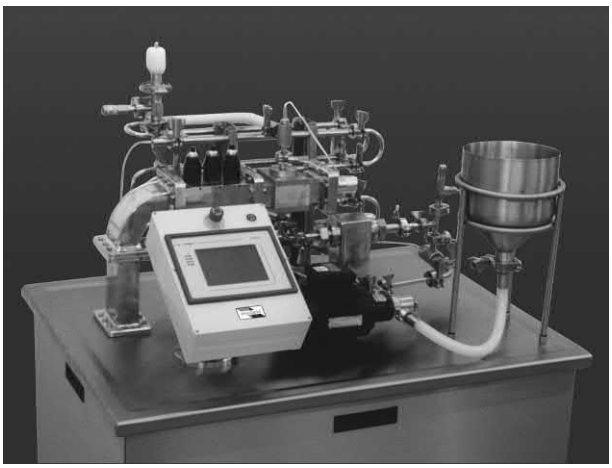

FIGURE 42.3 Multi-mode microwave tunnel cavity for packaged food pasteurization and sterilization.

42.8.3 Application to Food Systems

42.8.3.1 Milk

Milk is traditionally pasteurized in a heat exchanger before distribution. The application of MW heating to pasteurize milk has been well-studied and has been a commercial practice for quite a long time. The success of MW heating of milk is based on established conditions that provide the desired degree of safety with minimum product quality degradation. Since the first reported study on the use of an MW system for the pasteurization of milk [31], several studies on MW heating of milk have been carried out. The majority of these MW-based studies have been conducted to investigate the possibility of shelf life enhancement of pasteurized milk, application of MW energy to inactivate milk pathogens, to assess the influence on the milk nutrients, or to assess the non-uniform temperature distribution during MW treatment [31, 39–44].

Continuous-flow MW treatment has been proposed for milk pasteurization due to its potential advantages over the conventional tubular and plate heat exchangers. Continuous milk pasteurization at 2450 MHz using a simple waveguide heat exchanger was first reported by Hamid et al. [31]. Raw milk was passed through a glass tube fitted across a waveguide, and milk was exposed to MW energy during gravity falling. The plate counts were found to be negative while the temperature reached 82.2°C. Jaynes [39] developed an experimental continuous-flow MW pasteurizer using a Teflon tube (12 cm/0.635 cm) placed across a 2450 MHz MW guide. The system had a 15 s holding time. The adequacy of pasteurization was considered by the inactivation of phosphatase enzymes, standard plate, and coliform counts.

High-temperature short-time (HTST) sterilization of raw milk has also been tested under a MW field at 2450 MHz. A typical MW pasteurizer is shown in Figure 42.4. The process was done in a free-falling stream of milk with pressure application. Heating was reported to be extremely rapid with a temperature rise of 200°C; holding was less than second while the cooling was done by turbulent mixing with cold sterilized milk. However, the process was not considered economically feasible. Kudra et al. [40] used a domestic MW oven for continuous-flow pasteurization of milk and its constituents. The protein in milk was found to be the contributing component in dictating the heating pattern in milk pasteurization while the effects of fat and lactose were considered negligible. Lopez-Fandino et al. [45] reported the effects of thermal treatment of milk in a continuous-flow MW system by studying the

FIGURE 42.4 Microwave pasteurizer.

denaturation of β-lactoglobulin and the inactivation of alkaline phosphatase and lactoperoxidase using a modified MW oven at 2450 MHz. The results were compared with those obtained by conventional thermal treatment in a plate type heat exchanger, and the degree of inactivation caused by the thermal treatment in both cases was found to be similar. MW pasteurization of milk was reported to result in lower levels of denaturation of whey proteins compared to conventional thermal processes, and the denaturation of β-lactoglobulin was similar in both processes [46]. Moreover, the process yielded lower microbial counts and lower lactose isomerization. The sensory characteristics of MW-pasteurized milk were considered comparable to those achieved by traditional pasteurization after 15-day storage.

To overcome the non-uniformity of temperature distribution caused by MW heating, Coronel et al. [47] experimented on continuous-flow MW heating of milk at 915 MHz using a cylindrical MW applicator. The MW field inside the applicator generated a parabolic field distribution inside the tube for a fluid with constant dielectric properties, like those of milk at 25°C. The system was designed in such a fashion that the fastest moving particles reside at the center and would receive maximum power for a shorter period, whereas the slowest moving particles at the wall side would receive minimum power for a larger period. The system was reported to exhibit a relatively even distribution of temperature for milk, in the cross-sectional area of the tube at the exit of the applicator. Temperature distributions data revealed that the hottest temperature was found at the center of the tube while the cooler temperature was close to the walls of the tube.

42.8.3.2 Effect on Milk Nutrients

Milk is a rich source of vitamins, and heat treatment affects some of these nutrients. The effects of MW heating on several vitamins in cows' milk have been studied by many researchers [45]. Most studies report an insignificant loss in vitamin A, β-carotene, vitamin B_1, or B_2 in MW-pasteurized milk while losses of approximately 17% for vitamin E and 36% for vitamin C have been found. Sierra et al. [48] compared the heat stability of vitamins B_1 and B_2 in milk between continuous MW heating and conventional heating having the same heating, holding, and cooling steps. No significant losses in the vitamins were reported during MW heating at 90°C without holding period, while vitamin B_2 was found to decrease by 3–5% during 30–60 s holding. The authors concluded that the MW process does not offer any additional advantage concerning vitamin retentions as compared to a conventional heating process. MW heating of milk does not affect protein or fat components. Volatile components of conventionally treated and MW-treated (continuous-flow) milk have differed significantly.

42.8.3.3 Effect on Microbial Inactivation

The inactivation of *Streptococcus faecalis*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *L. monocytogenes* in milk by MW energy has been reported by Choi et al. [42, 43]. A complete inactivation was achieved when *Y. enterocolitica*, *C. jejuni*, and *L. monocytogenes* were heated at a constant temperature of 71.1°C at 8, 3, and 10 min using MWs with initial microbial loads of 10^6–10^7 CFU/mL [42, 43].

42.8.3.4 Fruit Puree and Juices

The inactivation of vegetative microflora in fruit products including purees and juices by MW heating, in general, does not pose any serious threat, and can be achieved by processing those products at a high temperature for a short time. However, some reports revealed that a few pathogens, namely *Salmonella enerica*, *E. coli O157:H7*, and *Cryptosporidium parvum*, have been associated with the foodborne illness in fruit juices. Pasteurization of fruit juices is traditionally carried out by a HTST heating process using a plate heat exchanger followed by a brief holding period and cooling. However, fouling is a major problem with the process. Inactivation of microorganisms and enzymes of fruit products, e.g., citrus juices by MW pasteurization, especially in continuous-flow systems, has created interest among juice processors due to lower thermal exposure, elimination of fouling in the pipeline, and retention of juice quality.

In orange juice, pectin methyl esterase (PME), an undesirable enzyme, causes spoilage and cloud loss during storage. Also, the enzyme is more heat resistant than spoilage microorganisms and, therefore, has been considered as an index of the adequacy of pasteurization. Nikdel et al. [49] described a continuous-flow MW system to pasteurize orange juice using PME inactivation and microbial count as indices. However, the work did not consider the time requirement for achieving the MW exit temperature, and come-up time (CUT) and come-down time (CDT) contributions were not considered in these studies. The inactivation of PME and *Lactobacillus plantarum* was found to be more pronounced using MWs as compared to conventional heating. The kinetics of PME inactivation in orange juice during MW heating in continuous as well as batch-mode processes were compared with those during conventional heating by Tajchakavit and Ramaswamy [26, 27], and they found largely enhanced inactivation of PME while employing MW heating. In a batch process, orange juice in glass beakers, with good mixing, was heated in an MW oven for a pre-selected time to achieve the desired

temperature. In the continuous-flow system, the juice was pumped through a helical glass coil placed inside the MW oven under full power heating conditions, and a target exit temperature was achieved based on juice flow rate and initial temperature. Under steady-state conditions, the increment of fluid temperature between inlet and outlet in continuous flow was found to be non-linear along the tube length. Under both batch and continuous-flow MW heating conditions, PME inactivation rate was significantly higher than in conventional thermal treatment at selected temperatures (D values at 60°C: batch MW = 7.37 s, continuous MW = 22 s, and conventional = 150 s). The authors claimed some enhanced-thermal effects during MW heating largely contributing to greater PME inactivation.

MW pasteurization of apple juice has also been investigated by several researchers [19, 45]. Tajchakavit et al. [50] studied the destruction kinetics of *Saccharomyces cerevisiae* and *L. plantarum* in apple juice under continuous-flow MW heating conditions and compared them with conventional batch heating in a water bath. The z values under MW heating for *S. cerevisiae* and *L. plantarum* were found to be 7 and 4.5°C, respectively, while the corresponding batch conventional heating values were 13.4 and 15.9°C, respectively. Microbial destruction thus was much more temperature-sensitive under MW heating than under thermal heating. Based on the computed D values, the authors again suggested some contributory enhanced effects to be associated with MW heating. However, Canumir et al. [51] reported that exposure of *E. coli* to MW treatments at 2450 MHz resulted in a reduction of the microbial population in apple juice and that the inactivation is solely due to heat. The pasteurization was carried out at different power levels (270–900W), and it was reported that a 2–4-log reduction in the microbial population was achieved at 720–900 W for 60–90 s with D values ranging from 0.42 min at 900 W to 3.88 min at 720 W. Recently, Gentry and Roberts [19] developed a continuous-flow MW pasteurizer using helical coils distributed through a large cavity oven to produce uniform and reproducible heating of apple cider. The process lethality of apple cider was verified by inoculation of *E. coli* 25992, and the 5D reduction was reported.

Piasek et al. [52] employed EnbioJet MW Flow Pasteurizer to examine the preservation of plant phytochemicals and bioactivity of anthocyanins-rich aronia and blue-berried honeysuckle juices. The juices were sterilized using the power supply of 63 A/20 kW and the temperature range of 90–135°C. The MW exposure time was 7 s. Compared to thermal treatment, a higher stability of phytocomplexes during processing was observed. In the same batches of juices subjected to heating at 100°C, the sharp decline of anthocyanin content accompanied by lowered antioxidant activity was noticed. The changes in chemical composition were reflected in altered biological activity. Both cytotoxicity and protection of DNA against oxidative damage were higher for microwaved juices than for juices processed by the conventional heating that caused degradation of bioactive phytochemicals.

Marszałek et al. [53] compared the quality and shelf life of strawberry purée processed by continuous MW heating at 90 and 120°C for 10 s to conventional thermal pasteurization at 90°C for 15 min during prolonged storage at 6°C. It was observed that MW heating at 120°C and conventional pasteurization were efficient for total microbial inactivation, which allowed for the storage of samples for up to 52 weeks. However, the sample treated at the lower temperature (90°C) during MW heating did not sufficiently reduce the total microbial counts in strawberry purée which resulted in shortening the shelf life to up to 44 weeks. Contrary to conventional pasteurization, MW heating could not completely achieve the inactivation of polyphenol oxidase (PPO) and peroxidase (POD). Furthermore, it was found that the PPO activity decreased about twofold during the storage of MW-heated samples while POD activity substantially increased in all the samples during 52 weeks of storage.

Benlloch-Tinoco et al. [54] evaluated the use of pasteurization units (PU) (Eqn. (14)) as a measure of the lethal effect during kiwifruit puree processing with the aim of comparing conventional (heating at 97°C) and MW (1000 and 900 W) technologies for POD and *L. monocytogenes* inactivation. The temperature profiles of the samples during processing were determined at different positions. The conventional heating mode required a significantly ($p < 0.05$) higher thermal load to achieve the pre-set level of POD inactivation (90%) in the kiwifruit puree than any of the MW treatments studied, irrespective of whether the comparison was carried out on the coldest or hottest spot of the sample.

$$PU = \int_0^t 10^{\frac{(T(t)-T_{ref})}{z}} dt \tag{42.14}$$

Franco et al. [55] studied dielectric properties and electrical conductivity of green coconut water (GCW) by open-ended coaxial probe technique at temperatures from 0 to 90°C and frequencies between 500 and 3000 MHz. At 915 MHz, ionic conduction shows an important role in MW heating, while both ionic and dipolar mechanisms were found at 2450 MHz, depending on temperature. The addition of sugars had a weak effect on polarization or loss. Component interaction significantly dropped the loss factor by 12% at 915 MHz and 8% at 2450 MHz.

42.8.3.5 Ready-to-Eat Meals

The frozen ready-to-eat food-processing industry is enormous and has been rapidly expanding over the years. The growth of the industry has been generated by the consumer demand for ready-to-eat meals which can be reheated easily before eating. The pasteurization schedule of ready-to-eat meals needs to be established through the same guidelines that are used for commercial sterilization and cannot be simply specified regarding a time–temperature combination. The pasteurization of ready-to-eat meals using MWs to enhance their shelf life has been recognized for many years, and the potential of the method has been verified in pilot-scale systems [56]. Pilot- and commercial-scale MW pasteurizers are available presently for this purpose. However, the adoption of the

technology by the food processing industry has been slow due to the uncertain trends in the markets for chilled foods with extended shelf life and also because of the technical limitations linked to the process. Ideally, the product should be heated to specified levels without over-cooking, then cooled quickly and properly stored and distributed. All along, the product should remain microbiologically safe while its shelf life is extended.

Various procedures for overcoming the technical problems have been considered including the use of a liquid circulating around the packs of food to restrict edge heating, pressurized systems for elevating temperatures or using partially open packs to prevent the bursting of packages, moving the waveguides, hot filling of product components to produce more uniform product temperatures, or incorporating metallic structures into the cavity to modify the field distribution. Many of these changes increase the complexity and cost of the equipment and may impede the flexibility of product types. Improving the design procedures for MW systems, including the design of the MW cavity, food packaging, and food composition, should lead to better processes. An efficient engineering approach requires methods that are capable of predicting the electric and magnetic field and temperature distributions in foods during MW heating. Heat processing at 80–85°C for a few minutes is considered sufficient, with a margin of safety, to inactivate vegetative pathogenic microorganisms such as *Salmonella* and *Campylobacter* but not bacterial spores. However, most bacterial spores do not multiply at low temperatures below 4°C. The growth from spores needs to be considered only when they are known to be present in the product ingredients and prolonged storage periods are expected.

Effect of various combinations of MW ovens (domestic, pilot-scale tunnel) and frequencies (2450, 896 MHz) have been studied for the pasteurization of ready-to-eat spaghetti bolognese meals in retail packaging to extend the shelf life [57]. It was reported that mean product temperatures above 80°C could be achieved using any of the systems but only the tunnel operating at 896 MHz heated all of the products in a pack above that temperature.

Ryynanen and Ohlsson [17] studied the importance of chemical and physical modifications in four-component chilled ready meals during uniform MW heating. The food component placement and geometry of products and packages were reported to play a significant role in providing MW heating uniformity of multi-component food systems. The temperature distribution could be balanced partly by taking advantage of edge and corner heating intensification. In contrast, chemical modifications, such as saltiness, did not notably affect the heating uniformity. However, interaction effects can sometimes be important.

42.9 STERILIZATION SYSTEMS

The pasteurization of packaged bread, pasta, and pizza have been reported. In some European countries, the whole loaf of the packaged bread is treated with MW energy to enhance the shelf life. MW-treated fresh pasta in packs further needs controlled atmosphere storage to increase the shelf life. MW processing for fresh filled pasta became common in Italy in the 1990s, and the technology has been applied to ready-to-eat meals, pasta-based products, and a variety of other foods. Some of the leading global food manufacturers apply the technology, including Unilever and Barilla SpA in Italy and Morinaga in Japan. However, these products have had limited success due to the excessive cost of the process.

In the 1970s Kenyon developed a high-quality shelf-stable ration as a replacement for the U.S. Army's C-ration by using a fiberglass-reinforced polyester pipe which was installed in a 10 kW MW oven. The system was similar to the equipment shown in Figure 14.3. A pair of butterfly valves at the product entry allowed pouches to be introduced into the processing system on a narrow conveyor belt through the MW field. The pouches dropped off at the end of the belt into a cold-water tank from which they could be removed periodically. Radiation heat losses were minimized by wrapping the pouches in MW-transparent insulation. However, the temperature difference from the edge to the center of the pouches was reduced to 5°C or less from 30°C or more. Another contemporary study of MW packaged food carried out by Alfa Laval, Sweden, was found to establish a decreasing temperature gradient from edge to center by introducing a cooling step to lower the temperature at the edges. Another modification of MW sterilizer was carried out based on MW heating of water by conveying packages of products through water; the water temperature was progressively adjusted by conventional heating means to provide edge heating control without expending too much energy in MW heating of the water. The system was installed in a Swedish food plant [6].

HTST processing of pouches by conventional retorting has clearly proven the benefit of such processing on some quality parameters. According to Ohlsson and Bengtsson [58], there is no reason to believe an MW HTST process would be any less successful. The authors [58] made a comparison of canning, retorting foil pouches, and MW sterilization of plastic pouches regarding the cook value (in an integrate value describing the effect of time and temperature on product quality). The quality of a variety of products processed using MW sterilization was superior. Typical commercial MW reheating and sterilization systems are shown in Figures 42.5 and 42.6.

Limited publications of data on processing two- and three-component ready meals are available. Processing meals presents a more complex problem—a differential heating pattern of various components. The problem could be solved by accounting for the energy requirements of each component. Several patented references are available on the topic while public information is lacking. Data available on the effect of storage time at room temperature on the quality factors of MW-sterilized foods are scarce. However, there are some results in which MW-sterilized (2450 MHz) vegetables were compared with foil pouch sterilization at 121°C and a frozen reference. The MW product was comparable with the frozen product for sensory characteristics even after 6 months at 25°C [59]. In another study with chicken à la King, the appearance

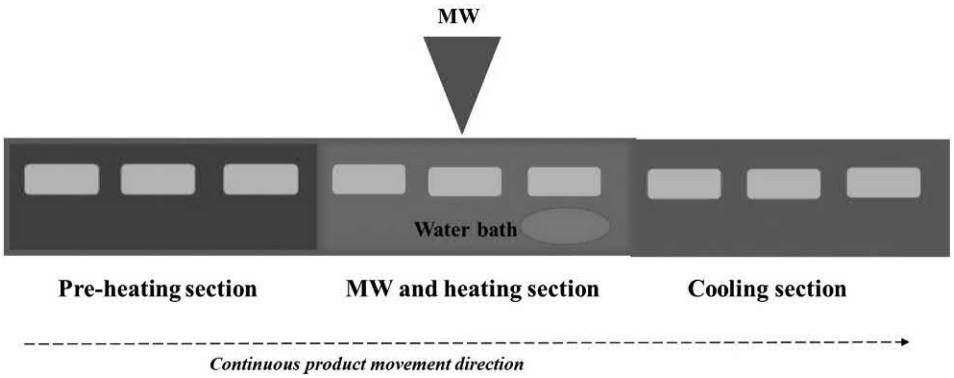

FIGURE 42.5 Pilot-scale MW-assisted pasteurization system (MAPS) for packaged food.

of MW-sterilized product compared favorably with the frozen reference even after 12 months' storage.

42.10 MARKER FORMATION AS AN INDEX OF MW STERILIZATION

Implementation of the MW sterilization process can vary significantly among process design and manufacturers. The application of the process in the food industry especially in the United States depends on the FDA approval of the dielectric sterilization process as a reliable procedure to establish and validate the required lethality for heat treatments to ensure microbiological safety. To design a new thermal process that ensures adequate sterility for shelf-stable foods, it is necessary to locate the cold-spot in packaged foods [60]. Once the cold-spot is determined, accurate time–temperature data can then be gathered from the slowest heated point and used for developing suitable thermal processes.

Numerous biological integrators have been found in the literature, and most of those have been used as relative indicators for food safety. None of those are adequate for the identification of the coldest point of thermally processed foods. Microbiological assays are good indicators to determine the effectiveness of a process; however, the procedure has limitations as the process is time-consuming, expensive, subject to recovery and contamination problems, and requires large population changes as evidence of the process. The use of effective chemical marker techniques developed by United States Army Natick Research Center has been reported to be an alternative technique to quantify the time–temperature history of food products to assess heating uniformity in sterilized foods. Three markers have been in use for food systems, namely: 2, 3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one (M-1), 4-hydroxy-5-methyl-3(2H)-furanone (M-2), and 5-hydroxymethylfurfural (M-3) [51]. The marker yield can be correlated with the time–temperature effect within food systems provided the kinetic information is obtained. The formation of intrinsic chemical markers is described as first-order reaction kinetics, taking into consideration an excess source of either the protein or ribose/glucose precursor in food materials [61]. Since both marker formation and bacterial destruction are functions of the time–temperature profile, verification of calculated marker formation using experimental studies is analogous to verification of bacterial destruction in sterilization. The marker formation kinetics of ham and whey proteins has been well compared to experimental and mathematical models by Zhang et al. [62].

The yield of M-2 in whey protein gels as model foods was used to quantitatively assess the heating uniformity of MWs

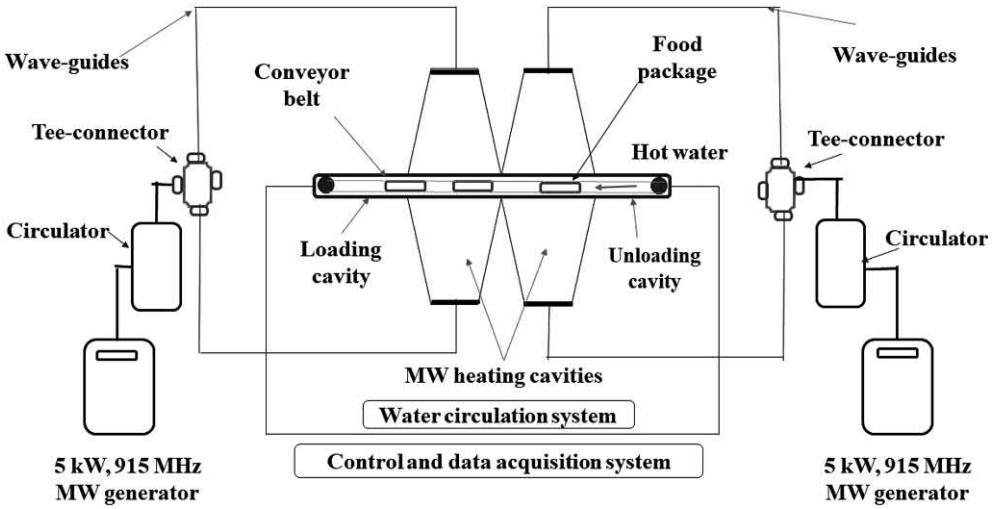

FIGURE 42.6 Pilot-scale MW-assisted sterilization system.

at 915 and 2450 MHz [63]. M-2 well predicted a HTST process, while M-1 was found to be more relevant for longer thermal processes [64]. Kim and Taub [61] evaluated the kinetics of M-1 in broccoli extract at sterilization temperatures (116–131°C) practiced in the food industry. Zhang et al. [62] evaluated the concentration of marker compound formed (M-2) during heating of whey protein concentrate solution and ham, and experimental data combined with numerical analysis were reported to result from an accurate and comprehensive study of the sterilization process. The marker yield increased beyond a temperature of 100°C, and the maximum was at 121°C. Lau et al. [64] studied the chemical marker formation of 4-hydroxy-5-methyl-3(2H)-furanone (M-2) in a model food system (20% whey protein gel) to identify cumulative time–temperature effects in high-temperature-short-time processes at 915 MHz. The formation of M-2 occurs from D-ribose and amines through non-enzymatic browning reactions and enolization under low-acid conditions (pH > 5). M-2 formation follows first-order reaction kinetics and can be used for determining the cumulative heating effect in a model food system subjected to MW heating.

However, the main limitation of using the chemical marker technique in real foods is the inconsistency of food composition in the food system leading to potentially large variations in the measurements of the marker yields. In addition, the heating pattern changes with food materials, placement of foods in ovens, and oven design, and therefore a combination of a coupled thermo-electromagnetic model along with experimental measurement of marker formation could provide a better picture of MW sterilization.

42.11 LIMITATIONS AND FUTURE OF MW HEATING

MW sterilization has been studied extensively for the academic and industrial sectors. However, the commercialization of the process has had only limited success [29]. The major drawback in the MW sterilization is the non-availability of actual temperature profiles. The measurement of temperatures at a few locations does not guarantee the real temperature distribution of the product during MW heating as the heating pattern can be uneven and difficult to predict, and can change during heating. Therefore, researchers in the field have found inconsistent outcomes.

Second, it is not always true that the MW-assisted process results in better quality retention of food products. The degradation kinetics of either quality, sensory, or nutritional aspects depend upon many factors like the nature of the food products, food geometry, dielectric properties, and oven designs as compared to conventional thermal processing. The dielectric properties of the food product significantly vary during heat processing and especially at or above 80°C for protein and starches, and simultaneously the heat absorption process. These changes in dielectric properties could affect the heating pattern qualitatively while such factors are not serious in conventional thermal processing. Coupling of heat transfer and electromagnetics could serve to account for changes in dielectric properties during thermal treatment [62].

The novelty of the MW sterilization process depends on the proper selection of equipment and packaging which could assure its success in food processing industries. Laboratory processing equipment is also essential for process refinement and to study the effect of process and storage time on product quality attributes, and microbiological safety factors. It is well-recognized that MW sterilization can produce high-quality shelf-stable food products. Only the most recent work had the benefit of suitable barrier packaging material. However, the earlier work recognized the need for suitable barrier material. Recently, few packaging material suppliers have shown serious interest in this process.

42.12 RECOMMENDATIONS FOR MW PASTEURIZATION AND STERILIZATION

Based on views and research outcomes of several experts in the field of MW technology applied to food, the U.S. Food and Drug Administration published the following recommendations in 2002 for better heat transfer and temperature management in MW heating:

1. Temperature distribution in food during and after MW heating is different from that using the conventional heating method. Therefore, the temperature should be measured with various techniques for a more reliable record of temperature distribution. The temperature should be measured in as many places as possible to predict more accurate information and time–temperature history of the product during MW thermal processing.
2. Information on the coldest point and its location are of primary importance for the microbial safety of sterilized food. As heating patterns can change dramatically for various food materials, different placements in the oven and different oven designs and, since the patterns can also change during heating, a combination of a coupled thermal-electromagnetic model complemented with experimental measurement of marker formation are needed for a comprehensive and repeatable MW sterilization.
3. To obtain MW heating uniformity of multi-component food systems is dependent on food component placement and geometry of products and packages. Placement has the most significant effect. The temperature distribution should be balanced partly by taking advantage of edge and corner heating intensification.
4. A combination of the thermal-electromagnetic model and marker formation kinetics should be used to describe MW sterilization in a comprehensive way. Coupling of heat transfer and electromagnetics is important to consider for significant changes in dielectric properties during the heating of foods. The model

predictions should be verified by obtained experimental data involving chemical marker yields that are functions of the time–temperature history of the material.
5. The time–temperature history and thus the sterilization vary spatially in a very significant way. Additionally, heating changes the relative spatial variation in sterilization. The spatial non-uniformity of sterilization and its transient changes can be improved significantly by changing the material's dielectric properties, which are a function of its composition. The effect of salt content was found to be particularly pronounced.
6. To improve the MW heating efficiency and desirable sensory characteristics of foods, the combination of MWs with other modes of heating such as infrared heating (IR) and jet-impingement can be used.
7. Applying MW energy at a lower frequency, e.g., 900 MHz, would show higher penetration depths in materials such as foodstuffs.

42.13 CONCLUSION

MW energy has advantages over conventional heating. The application of MW energy for pasteurization and sterilization has been studied for about half a century with some commercial success. Some researchers have claimed non-thermal or enhanced thermal effects to be associated with MW heating on the destruction of microorganisms and inactivation of the enzyme, but the issue remains controversial. Continuous-flow microwaveable pasteurizers could be used for milk and juice processing. MW pasteurization of ready-to-eat meals has also been found to be a commercial success in the European countries while US industries are still reluctant to adopt the technology. The replacement of conventional heating by MW energy source is not possible without fully understanding the real heating and inactivation mechanisms, temperature distribution in multilayered foods, and other critical factors. The qualitative and significant change in heating pattern has to be taken into consideration in the calculations of marker yields by coupling the electromagnetics with energy transfer in MW sterilization. Currently, more emphasis has been placed on the sterilization of solid foods using MW energy. Commercial-size MW equipment is now readily available for pasteurization and sterilization applications. Some reasons cited for the lack of commercial success in operation are complexity, high expenditure, non-uniformity of heating, inability to ensure sterilization of the entire package, lack of suitable packaging materials, and unfavorable economics when compared to prepared frozen foods in developed countries.

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43 Ultrasound in Food Processing and Preservation

P. J. Torley, T. T. Truong, and B. Bhandari

CONTENTS

43.1 INTRODUCTION

The effects of high-power ultrasound waves on the physical, biochemical, and microbial properties of foods have attracted a great deal of interest in recent years. This is because ultrasound can produce a variety of effects depending on the ultrasound wave characteristics and product characteristics. Ultrasound can be used to characterize the products as non-destructive or to produce physical transformations. Ultrasound

is important in many situations, including natural systems (e.g. communication between rats at ultrasonic frequencies [1]; echolocation by bats and dolphins; detection of ultrasound signals by fish and moths to avoid predators [2, 3]), engineering design to minimize cavitation (e.g. erosion of impellers in pumps [4], non-destructive evaluation of manufactured products [5], enhancing the efficiency of industrial processes [6, 7]), detection of underwater vessels and oceanography [8], identifying fish stocks [9], medical imaging [10], surgery [11], and physiotherapy [12]. Many of the techniques used in these situations have parallels in food processing, where ultrasound can either be used to monitor processes while minimizing any effects on product characteristics, or to become directly involved in the process, helping to bring about physical transformations in the product. This chapter will focus on the latter aspect, and will include an overview of the history of ultrasound development, the basic principles underlying the effects of sonication on properties of food systems, and the use of ultrasound in food processing, particularly microbial inactivation, heat and mass transfer, and homogenization.

With such a broad range of applications, the history of ultrasound research, development, and application is extremely complex. Systematic studies of physical, chemical, and biological effects produced by ultrasound began in the early 1900s. In 1917, Lord Raleigh developed a mathematical model for cavitation bubble collapse while investigating the problem of high-speed propeller erosion [13]. Also in 1917, Langevin discovered that sound rays killed fish while studying sonar for antisubmarine warfare. The late 1920s was also an important time in ultrasound research with potential applications in the food industry, with a number of important ultrasound effects [14, 15]. During this time various researchers reported that ultrasound could be used to rupture microorganisms, emulsify oil and water, atomize liquids, cause agitation inside individual plant, animal, and amoebae cells, accelerate chemical reactions, and degas liquids. Mechanisms to explain the effects of ultrasound were identified, including heating, agitation, aggregation, and cavitation [14]. There are a number of reviews available that can be consulted for a more complete history of ultrasound development [13–17].

43.2 BACKGROUND

43.2.1 Sound Waves

Ultrasonic waves are similar to sound waves, but their frequencies are far too high for perception by the human ear. Transmission of sound occurs due to ordered and periodical movements of the molecules of the media, with motional energy passed on to adjacent molecules without transfer of matter. Typically, the range of frequencies perceived by humans is 20 Hz to 20 kHz, while ultrasound is from about 20 kHz to 1.2×10^{10} kHz (the highest frequency that can be transmitted by solids or liquids). Ultrasound has the properties of sound waves, such as reflection, interference, adsorption, and scattering, and can be propagated through solids, liquids, and gases [18, 19].

Sound waves can be either parallel or perpendicular to the direction of travel through the material, and are respectively termed as longitudinal and transverse waves (shear waves) (Figure 43.1). In transverse (shear) waves, particle motion is perpendicular to the direction of wave propagation. As liquids and gases do not support shear stress under normal conditions, transverse waves can only propagate through solids. The velocity of these waves depends on the material (Table 43.1) and is relatively low as compared to longitudinal waves.

In longitudinal waves, the direction of particle motion is the same as the wave motion. These waves are capable of traveling in solids, liquids, or gases, and thus are widely used in ultrasonic applications. Longitudinal waves have a short wavelength with respect to the transducer dimensions, producing sharply defined beams, and have a high velocity (Table 43.1). The longitudinal velocity is dependent on the state of the material and so can be used to follow processes such as freezing of orange juice [20], meat [21], or solidification of fats [22]. It is also sensitive to differences in structure, such as fiber orientation in meat [23] and comminute meat composition [24].

43.2.2 Wave Characteristics

The fundamental parameters that characterize ultrasound waves are velocity (c), wavelength (λ), frequency (f) amplitude (A), and intensity (I). Velocity is the propagation speed of a sound in a medium and is related to wavelength and frequency:

$$c = \lambda f \tag{43.1}$$

It depends on the physical properties of the medium through which waves propagate. For solids and liquid, velocity is related to density (ρ) and Young's modulus of a solid, or bulk modulus of elasticity for a liquid (K):

$$c = \sqrt{\frac{K}{\rho}} \tag{43.2}$$

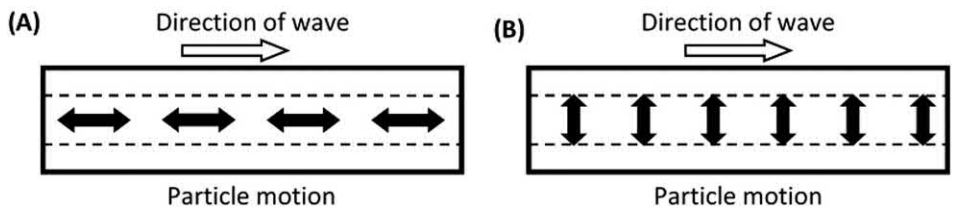

FIGURE 43.1 Types of sound wave: (A) longitudinal waves (B) transverse (shear) waves.

TABLE 43.1
Velocity of Transverse and Longitudinal Sound Waves in Different Materials

Material	Wave Type	State	Velocity (m/s)
Air	Longitudinal	Gas, 20°C	344
Aluminum	Longitudinal	-	6374
	Transverse	-	3111
Water	Longitudinal	Water Vapor	500
		Water, 25°C	1498
		Ice	3760
	Transverse	Ice	2000
Orange juice [20]	Longitudinal	Liquid, 20°C	1540
		Frozen, −20°C	3310
Beef (direction of ultrasound signal either parallel or perpendicular to muscle fiber orientation) [23]	Longitudinal	Warm, 37°C	1595
		Perpendicular	1605
		Parallel	1525
		Chilled, 0°C	1531
		Perpendicular	2870
		Parallel	2930
		Frozen, −9°C	
		Perpendicular	
		Parallel	
Fat content of meat mixtures [24]	Longitudinal	100% lean meat	1543
		50% lean meat/50% fat	1584
		100% fat	1617
Olive oil [22]	Longitudinal	Liquid, 60°C	1490
		Solid, −30°C	1990

For gases, the relationship includes the pressure (P) and the specific heat at constant pressure (C_p) and specific heat at constant volume (C_v):

$$c = \sqrt{\frac{C_p P}{C_v \rho}} \tag{43.3}$$

Wavelength is the distance between adjacent wave crests (Figure 43.2), while frequency refers to the number of wave crests that pass a point in a unit time, and resembles the vibration of the wave generator. The ultrasound wavelength at 20 kHz frequency in water at 25°C is around 7.5 cm, whereas in more dense systems (such as in muscle or bone) it will be higher, and in the air it is around 1.65 cm.

Amplitude is the height of the wave, and it determines the wave strength. Amplitude is related to the energy contained in the wave:

$$E \propto A^2 \tag{43.4}$$

Amplitude refers to the motion of the ultrasonic source, the motion of the receiver of the sound, or the motion of the medium through which the sound wave is passing. Intensity is a measure of the flow of acoustic energy through a unit area of the medium in a unit time. As sound waves pass through any real medium or biological tissue, the intensity of the signal decreases with distance of travel due to scattering of the sound waves and absorption of part of the sound energy

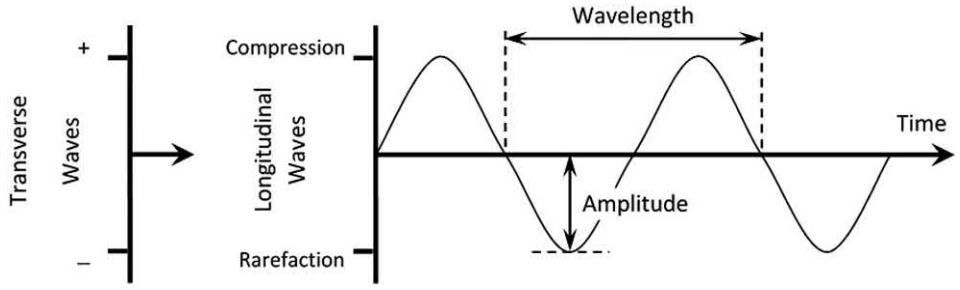

FIGURE 43.2 Transverse and longitudinal wave characteristics.

by the material. The absorption of sound is due to a number of mechanisms: viscous losses, heat condition losses, and losses due to molecular exchange of energy [18]. Scattering is caused by interfaces between materials with differing acoustic impedances (sound reflection properties). The heterogeneity in a material can greatly increase attenuation when there are large differences in the density of the two materials, such as gas bubbles suspended in a starch water gel. The decrease in intensity (I) follows an exponential pattern which depends on the initial intensity at the transducer (I_o), distance from the transducer (x), and the amplitude attenuation coefficient of the material (α):

$$I = I_o e^{-2 \alpha x} \quad (43.5)$$

The sound energy absorbed is converted to heat causing an increase in temperature. The heat production (Q_v) is related to the intensity of the sound (I) and the amplitude attenuation coefficient of the material (α):

$$Q_v = 2 \alpha I \quad (43.6)$$

The amplitude attenuation coefficient varies between materials, and with the frequency of the sound. Generally, the absorption coefficient increases with increasing frequency and in biological systems can vary markedly between materials [25].

43.2.3 Cavitation

In liquid media, the best-known effect of ultrasound is cavitation. When an intense sound wave passes through a liquid, it creates regions of compression (positive pressure) and rarefaction (negative pressure). If the negative pressure during rarefaction is high enough, a cavity or bubble can be formed in the liquid. There are two main types of cavities: transient (also called "inertial") and stable (also called "non-inertial"). Each of them demonstrates a different type of behavior of a gas bubble that is subjected to an ultrasonic field [26].

Transient cavitation occurs when a cavity that is exposed to ultrasound vibration progressively increases in size over a number of compression and rarefaction cycles until it reaches a size where it collapses violently [27]. During cavity collapse, very high, but localized, temperatures, pressures, and cooling rates occur. A theoretical study has estimated the temperature created as being up to 10,000 K, pressures of up to 100 MPa (though an experimental study suggests 0.01 to 0.5 MPa [7]), and cooling rates in the order of 10^{10} K/s [28]. It is also reported that an electrical field can occur at the interface when a cavity is fragmented [29].

Long-lived gas bubbles are called stable cavities and will exist for many compression and rarefaction cycles [30]. They are produced at relatively low ultrasound intensities (1 to 3 W/cm^2) and will oscillate for a number of cycles, often nonlinearly about some equilibrium size without collapsing [31]. While the conditions within stable cavities are not as extreme as transient cavities, relatively high pressures and temperatures (estimated at about 1650 K) still occur, which allows them to influence chemical reactions.

Whether or not cavitation occurs depends on a variety of factors including the frequency of the vibration (increasing frequency increases the power required to produce cavitation), intensity of the vibration (related to amplitude), solvent viscosity, surface tension and vapor pressure, attenuation of the vibration by the medium (greater attenuation at higher frequency), the presence of gas bubbles as cavitation nuclei, and the ambient temperature and pressure [31].

43.2.4 Liquid Flow and Bubble Movement

Both the large and localized agitation produced by ultrasound is of considerable practical importance. Ultrasound agitation is the origin of at least part of the beneficial effect of ultrasound in process, such as heat and mass transfer, emulsification, cleaning of surfaces, and polymer degradation.

Microstreaming occurs due to the growth and shrinkage of the cavitation bubbles during cycles of rarefaction and compression [32]. The rapid movement of liquid caused by changing bubble size causes shear; however, the effect is limited to about one bubble diameter. Microstreamers are bubbles that form at a nucleation site and then move through the acoustic field to a pressure antinode (if the bubble diameter is less than resonance size) or pressure node (if larger than resonance size) [26, 32, 33]. Resonance size is determined by the properties of the liquid and acoustic wave frequency, and it is the size where the applied frequency produces large changes in bubble diameter. Owing to differences in the speed of bubble movement depending on whether they are in the compression or rarefaction part of the acoustic wave, microstreamers are able to move faster than the average liquid velocity.

When a transient cavitation bubble collapses, there is a rapid inflow of liquid from all sides causing high localized shear rates and shockwaves in the surrounding liquid [31]. If a transient cavitation bubble collapses when it is close to a solid surface a micro-jet is formed. In this situation, the solid surface means the liquid will not be able to flow in from all sides, creating a non-symmetrical flow that directs a micro-jet of liquid at the solid surface [26, 31, 34]. The jet of liquid has a relatively high velocity (about 100 m/s), though it is a relatively short-range effect (about a bubble diameter) [32]. Depending on the circumstances, micro-jets may have advantageous effects (e.g. cleaning surfaces, pitting increasing surface area for reaction), while in other situations they may be damaging (e.g. pitting of ultrasound probe tips, erosion of propellers). An analogous situation occurs at the interface between two immiscible liquids, where micro-jets can produce an emulsion.

There can also be movement of bubbles in acoustic fields due to the density difference between the gas bubble and the liquid medium. When the cavity forms, gas can progressively diffuse into it from the surrounding medium, or the bubble

can coalesce with other bubbles, progressively increasing in size until it floats to the surface. This is the basis of ultrasound degassing of liquids [35].

43.2.5 Sonochemistry

Sonochemistry is the term for chemical reactions performed under an acoustic field, commonly ultrasonic. The field has developed as it has been found that ultrasound produces a range of effects on chemical reactions in treated materials, including free radical formation, increased reaction rates, less extreme processing conditions (e.g. lower temperature, pressure), reduced induction period, easier initiation of difficult reactions, improved catalyst efficiency, permitting the use of less refined raw materials, reduced number of processing steps, alteration of the reaction pathway, and sonoluminescence [31, 33, 36, 37]. Sonoluminescence is the light emitted during the collapse of either stable or transient cavities due to the generated pressures and temperatures [38].

Within the collapsing cavity, there are extreme pressures, temperatures, and cooling rates, along with gas or vapor from the liquid medium. These conditions are capable of creating hydroxyl radicals from water, which can react with other chemicals in the cavity, or diffuse into the liquid medium where they can react with other compounds. Other reactions are possible if there is a volatile compound present in the liquid medium. The volatile compound can diffuse into the cavity during expansion, and can undergo chemical reactions during collapse of the cavity.

In addition to chemical reactions caused by the diffusion of reactive species or reaction products into the liquid medium, cavity collapse can affect the chemical reactions in the liquid medium. The high shear created by cavity collapse may also cause breaks in polymer chains, or increase reaction rates due to an increase in the kinetic energy of molecules, or alter interaction within the solvent.

Ultrasound waves can alter chemical processes at surfaces, with different effects depending on whether they are large, small, or liquid surfaces. There can be mechanical damage to the solid material, with shock waves and micro-jets causing surface damage including pitting of solid surfaces, fragmentation of brittle materials, deaggregation of groups of particles, and high-velocity collisions between small particles accelerated by ultrasound causing in some instances abrasion and in others, fusion. Such changes can alter chemical reactivity, for example through increased surface area, the removal of contamination from the solid surface such as oxidation from metals. With immiscible liquids, cavitation at their interface can create emulsions that greatly increase the area of contact between the two materials.

While the main focus of sonochemistry is the cavitation-related effects, the non-cavitational effects of ultrasound can play a role in chemical reactions. Ultrasound agitation of liquids improves mass transfer, and heating of the material due to energy absorbed from the ultrasound waves can increase the rate of chemical reactions.

43.3 ULTRASOUND INSTRUMENTATION

43.3.1 Transducers

A transducer is a device that converts one form of energy into another form. In ultrasonic applications, the transducers are designed to convert mechanical or electrical energy into high-frequency sound. There are two main types of transducers: mechanical and electroacoustic [7]. Mechanical transducers rely on the flow of either a liquid or gas through a siren, rotor, turbine, or whistle to generate ultrasound. Electromechanical transducers are widely used in modern ultrasonic applications and are based on the inherent electrostrictive phenomenon in certain materials to produce piezoelectric or magnetostrictive transducers [7].

43.3.2 Ultrasonic Equipment

There are different types of ultrasonic apparatus commercially available for small- or large-scale power ultrasound applications, including whistle reactors, ultrasonic baths, and probe systems [31, 35]. A whistle reactor uses a mechanical ultrasonic source that relies on the stream of liquid flowing past a metal blade to cause vibration [31, 35]. The frequency of the vibration depends on the liquid flow rate, with flow rates high enough to be able to generate ultrasound which can cause cavitation in the liquid. These sorts of systems can be used for high-power liquid processes such as homogenization, emulsification, and dispersion.

Ultrasonic baths are cheap, simple, and versatile, being made up of a metal bath with one or more transducers attached to the walls of the tank [31, 35, 36]. Items can be directly immersed in the bath for ultrasound treatment, though the maximum power input is generally relatively low (about 1–5 W/cm^2).

Probe systems consist of a metal horn coupled to an ultrasonic transducer, with the metal horn used to amplify the vibration produced by electrostrictive material (normally piezoelectric) in the transducer [7, 27]. Amplifying the vibration produced by the transducer is necessary as the amplitude of waves produced by piezoelectric materials is too small to have a useful effect. An appropriate horn design increases the amplitude of the vibration at the face of the horn. Probe systems have the advantages that they can be placed directly in or against the material being processed, their power can be controlled, and they can produce ultrasound intensities of up to several hundred W/cm^2 [30, 35, 36]. Disadvantages of the probe system include erosion of the horn tip by cavitation, free radical formation, and heating of the material exposed to ultrasound. Tip erosion by cavitation can cause contamination of the material by metal from the horn as well as a gradual change in horn length which will affect its efficiency.

A number of systems have been developed to overcome some of these limitations, including the cup horn, flow cell, and tube reactor. The cup horn system contains an ultrasound probe that incorporates a cooling system [36], with the cooling liquid (typically water) used as the coupling medium to transmit vibrations from the probe tip to the reaction vessel.

This approach allows better temperature control than bath or simple probe systems, though the reaction vessel size is limited and power input into the reaction vessel is reduced compared to a probe placed directly in the material being processed. The flow cell is a continuous system where the ultrasound probe is placed directly in a liquid that is being pumped through a cell, with treatment only occurring while the liquid is inside the cell [31, 36]. This allows high-intensity treatment and control over the temperature rise by controlling the residence time of the liquid. In tube reactors, the liquid is treated with ultrasound as it is pumped through a zone fitted with some form of ultrasound generator [31].

43.4 ULTRASOUND IN FOOD PROCESSING

The application of ultrasound in food processing has been of interest for many years, with researchers investigating its potential as a means of monitoring a process or product, or as a way of altering the properties of a process or product. Low-power, high-frequency ultrasound (<1 W/cm^2; >100 kHz) is normally used to monitor food products or processes. The use of ultrasound monitoring has been evaluated in a wide range of food systems including meat, fats and oils, milk, bread, fruit, and sauces [18, 19, 25], with various parameters, particularly velocity (m/s), attenuation (dB/m), and impedance (kg m/s), or related parameters, used to measure characteristics such as composition, phase changes, and particle size distribution.

High-power, low-frequency ultrasound (10 to 1000 W/cm^2; 20 to 100 kHz) is normally used to alter the properties of a material or affect the progress of a process. It does this through physical, chemical, and mechanical effects (Table 43.2). Ultrasound can affect food properties; however, it is common to use it in combination with other processing technologies to improve the efficiency of the process.

43.5 ANTI-MICROBIAL EFFECTS

The use of ultrasound as an antimicrobial treatment, either alone or in combination, has been of interest for many years for use in food products, or to remove contaminating microorganisms from foods and processing surfaces [39–44]. The antimicrobial effect of ultrasound is largely due to the localized, but extreme, pressures and temperatures produced during cavitation leading to damage to cell walls [41], with possible contributory effects due to direct thermal effects from the localized heating, production of free radicals causing damage to DNA, and microstreaming causing thinning of cell membranes [39, 40, 42].

43.5.1 Sensitivity of Different Microorganisms

Ultrasound treatment has been shown to be an effective antimicrobial treatment, destroying a variety of microorganisms, including bacteria [40, 45, 46], bacterial spores [47–49], yeast [50–52], fungus [45, 53, 54], fungal spores [55], protozoa [56], and viruses [45]. Comparative studies of the sensitivity of different microorganisms show the effectiveness of ultrasound varies widely, with even closely related microorganisms showing differences in sensitivity [57, 58]. In general, spores [46–49, 59] and some viruses [45] are difficult to inactivate with ultrasound. Gram-positive bacteria are less sensitive to ultrasound than Gram-negative bacteria [46, 60, 61], though no difference between Gram-positive and Gram-negative

TABLE 43.2
Some Applications of High-Power, Low-Frequency Ultrasound in the Food Industry

Application	Description	References
Anti-microbial effects	Microbial destruction, microbial removal from surfaces	[39–42, 68, 69, 237–240, 70–73, 75–77]
Heat transfer	Increase the rate of freezing, thawing, and cooking	[178–180, 182–184, 187–189]
Mass transfer	Increase the rate of mass transfer in drying (solid, liquid, and osmotic drying), brining, membrane separation, dewatering, and bed filtration	[78–93, 96–99, 101–105, 109, 115–117, 181, 241, 242]
Meat processing	Meat tenderization	[104, 105, 183, 184, 224–227, 229–231, 243]
Homogenization, emulsification, and encapsulation	Homogenize and emulsify milk, mayonnaise	[35, 63, 207, 219, 220, 244–247]
Fermentation and ageing	Increase rate of fermentation and ageing (e.g. wine)	[248–253]
Crystallization	Control of nucleation and crystal growth	[6, 35, 131, 254]
Cutting	Cut fresh and frozen food products, including composite or multilayer foods	[255]
Defoaming, defrothing, and degassing	Defoam carbonated drinks, beer, and other liquids during canning; defoam microbial fermenters; remove dissolved gasses from liquids	[7, 35, 90]
Cell disruption and extraction	Enhance extraction of compounds (e.g. enzymes, proteins, fruit juices, essential oils)	[13, 53, 256–258]
Enzyme activity and protein denaturation	Enzyme inactivation; protein denaturation; enhance enzyme activity	[196–200, 202, 205–209, 216, 217, 259, 260]
Polymerization and depolymerization	Polymerization and depolymerization of polymers	[28, 30, 261]

bacteria has been found [45]. It has also been observed that rod-shaped bacteria tend to be more susceptible than coccus-shaped bacteria [61], and that larger cells are more susceptible than smaller cells [42].

43.5.2 Effectiveness as an Anti-Microbial Treatment

While ultrasound used by itself can kill microorganisms, it is a relatively inefficient process, with extended processing times required to produce a significant reduction in microbial numbers. As a result, research into ultrasound as an antimicrobial treatment has largely focused on combining ultrasound with other treatments, particularly heat treatment (also termed thermosonication), pressure treatment (manosonication), and combined heat and pressure treatments (manothermosonication) [39, 42, 44], with less attention paid to its use with other treatments such as pulsed electric fields, antimicrobial chemicals, and pH [43, 46].

The lengthy processing required to destroy some microorganisms and particularly spores at temperatures well below temperatures used for conventional thermal treatment makes ultrasound an unattractive treatment. The D value of vegetative *Staphylococcus aureus* treated with ultrasound (20 kHz, 150 W, 40 ml sample volume) was 36.5 minutes at 11.2°C in phosphate buffer, and 187 minutes at 13.5°C when suspended in ultraheat-treated milk [62].

Ultrasound treatment of bacterial spores can have little effect at low temperature, with no reduction in *Bacillus stearothermophilus* spore numbers by an extended ultrasound treatment at 12°C (20 kHz, 120 W, 50 ml sample volume), though there is the release of low molecular weight materials [49]. *Clostridium sporogenes* and *Bacillus cereus* spores suspended in Ringer solution survived sonication (20 kHz, 500 W, 35°C) for 30 minutes, though combining ultrasound and 6% H_2O_2 proved an effective treatment [46]. Ultrasound was also reported to have an insignificant effect on the survival of *Clostridium botulinum* spores in honey [59].

Ultrasound produces a variety of effects that can be applied in dairy processing [63]. One effect that has been examined closely is its ability to inactivate microorganisms when used in combination with a heat treatment to give an effective method for the destruction of microorganisms present in milk [60, 62, 64, 65].

43.5.3 Ultrasound Combined with Heat and Pressure

Combining ultrasound with either pressure, temperature, or pressure and temperature treatments has proven a particularly popular approach as the length of ultrasound treatment can be substantially reduced [39, 42, 44]. Simply combining ultrasound with mild heat treatments has been found to increase the rate of microbial inactivation in a variety of microorganisms. *Streptococcus faecium* and *Streptococcus durans* had D values at 62°C (heat only) of 11.2 and 10.3 minutes respectively, while simultaneous heat and ultrasound (62°C, 20 kHz, 160 W) reduced D values by 84% and 91% respectively [58]. Yeast cells are destroyed more rapidly by a combined ultrasound and temperature treatment [52]. The D time for *Saccharomyces cerevisiae* was reduced by up to 63% by combined ultrasound (20 kHz, up to 180 W, 300 ml sample volume) and heat treatment (50°C or 55°C) compared to heat-only treatment [52]. The combined treatment was also successful with bacterial spores. The D value of *Bacillus subtilis* spores at 100°C was reduced by 38 to 84% (depending on strain and medium) by a combined heat and ultrasound treatment [47].

A limitation of combined ultrasound and thermal processing is that its effectiveness decreases with increasing temperature, as the increase in vapor pressure and the decrease in liquid surface tension and viscosity [47] reduce the cavitation effect [42]. To overcome this problem, processes combining ultrasound and pressure treatment, or ultrasound, pressure, and thermal treatments have been developed [42]. By pressurizing the material, cavitation can occur at higher temperatures, increasing the efficiency of microbial inactivation.

Even at temperatures well below the boiling point of the medium, using pressure during ultrasound treatment will affect microbial destruction. When different bacteria were treated by ultrasound (20 kHz, 117 μm amplitude, 40°C), increasing the pressure from 0 to 400 kPa produced substantially different decreases in D value for *Streptococcus faecium* (D value about 11 minutes at 0 kPa, 5 minutes at 100 kPa, 1.8 minutes at 400 kPa), *Listeria monocytogenes* (3.5, 2, and 0.8 minutes), *Salmonella enteritidis* (2, 1, and 0.4 minutes), and *Aeromonas hydrophila* (1.3, 0.8, and 0.4 minutes) [61]. However, when the results are expressed as a percentage, the variation in the decrease in D value is somewhat smaller, with a decrease in D value of 39 to 55% between 0 and 100 kPa, and 72 and 84% between 0 and 400 kPa [61].

Even closely related microorganisms show some differences in response to combined pressure and ultrasound treatment. When three *Salmonella* serotypes were ultrasound treated (20 kHz, 117 μm amplitude) at 40°C, increasing pressure from 0 to 50 kPa, the D value was reduced by 24 to 48% (depending on serotype), though at higher pressures the variation between serotypes was smaller. Increasing treatment pressure from 0 to 250 kPa decreased D value by 65 to 72% [57].

Combining pressure and thermal treatments further increases the antimicrobial effect of ultrasound. A combined pressure, thermal, and ultrasound treatment (20 kHz, 117 μm amplitude, 175 kPa) of *Salmonella enteritidis* gave D values that were lower than a combined thermal and ultrasound treatment at the same temperature [66]. The improvement from the combined pressure, thermal, and ultrasound treatment gave D values 68 to 91% lower at or below 60°C than the combined thermal and ultrasound treatment, but only 0 to 44% above 60°C.

The sensitivity of bacterial spores can also be increased by combined pressure, thermal, and ultrasound treatment. When *Bacillus subtilis* spores were pressure and ultrasound treated (200 kHz, 117 μm amplitude, 300 kPa) at 55°C and 70°C, only about 20% of the spores survived, while the heat-only treatment saw about 100% survival at the same temperatures [48].

43.5.4 Processing Medium

The processing medium can play an important role in determining the effectiveness of ultrasound as an antimicrobial treatment. When *Staphylococcus aureus* suspended in phosphate buffer received combined heat and ultrasound (20 kHz, 150 W, 40 ml sample volume) treatment, the D value was reduced by 60 to 65.5%, while the same treatment was less effective when suspended in milk, reducing the D value by 41 to 47% compared to a heat-only treatment [62]. A similar effect was seen with *Salmonella typhimurium*, where the number of survivors after ultrasound treatment was substantially higher in liquid egg (50% survival after treatment at 20°C for 15 minutes, 32% at 40°C) than skim milk (87% at 20°C, 19% at 40°C) or brain heart infusion broth (6% at 20°C, 16% at 40°C) [64].

Water activity can affect the effectiveness of ultrasound, with ultrasound proving more effective at higher water activity. When *Salmonella enteritidis* was treated with ultrasound (20 kHz, 117 μm amplitude, 58°C), the D value increased with decreasing water activity, from 0.22 minutes at a water activity above 0.99, to 1.53 minutes at water activity of 0.98 and 4.56 minutes at a water activity of 0.96 [66]. A similar pattern of increasing D value with decreasing water activity was also seen in samples exposed to a combined pressure, thermal, and ultrasound treatment (20 kHz, 117 μm amplitude, 175 kPa) [66]. While the water activity was not reported, incorporating 57% sucrose into the broth used to suspend *Listeria monocytogenes* before pressure and ultrasound treatment (20 kHz, 117 μm amplitude, 200 kPa, 40°C) increased the survival time, though adding 3% NaCl had no effect on survival time [67].

The pH of the medium can alter the effect of ultrasound treatment, with decreasing pH tending to reduce the survival of bacteria and yeasts [43]. Differences in the pH range of the ultrasound medium may help explain the relatively small variation in ultrasound's effectiveness against *Escherichia coli* suspended in UHT milk (pH 6.7), carrot juice (pH 5.9), or phosphate buffer (pH 7.0), and greater variation for *Lactobacillus acidophilus* suspended in either orange juice (pH 3.7) or phosphate buffer (pH 7.0) [65]. The survival of *Listeria monocytogenes* also decreased with a decrease in medium pH when treated with ultrasound (20 kHz, 117 μm amplitude, 200 kPa, 40°C), going from about 3.7×10^5 cfu/ml to 2.6×10^3 cfu/ml at pH 7, and from about 3.8×10^5 cfu/ml to 6.6×10^2 cfu/ml at pH 4 [67]. However, the decrease in pH had a much greater effect on the survival of *Listeria monocytogenes* when the samples were treated with heat (62°C) instead of ultrasound.

Differences in the viscosity of different mediums may explain some of the differences seen. The relatively higher survival of microorganisms in more viscous liquids is observed due to their high viscosity, protecting the microorganism by reducing cavitation. Ultrasound treatment was much less effective at reducing the level of *Salmonella eastbourne* suspended in milk chocolate than in peptone water. Other studies have shown that more viscous media can improve microbial survival, with slower destruction of *Bacillus subtilis* spores in glycerol than milk [47], and in egg than other media [57, 62, 64].

43.5.5 Decontamination of Food Surfaces

Raw fruit and vegetables used in some types of minimally processed foods have spoilage and possibly pathogenic microorganisms adhering to or entrapped in their surfaces. It is common to wash in water or water containing a sanitizer (typically 50–100 ppm chlorine) to reduce this microbial load. However, effective sanitizing is difficult as the bacteria can be difficult to destroy or remove as they are attached or entrapped in the uneven surface of the plant matter. Ultrasound can be incorporated in the sanitizing process to improve the effectiveness of the washing process, with combined ultrasound (32–40 kHz, 10 W/L of water) and chlorinated water giving a larger reduction (1.7 logs) in *Salmonella typhimurium* numbers on iceberg lettuce than ultrasound alone (1.7 logs), chlorinated water alone (1.6 logs), or washing in sterile water (0.7 logs) [68]. Larger-scale trials found that the product (parsley, strawberries, cabbage, and lettuce) and washing medium (water only, chlorinated water, or chlorinated water with a surfactant) had an effect on decontamination, while ultrasound frequency (0 kHz, 1.0 log reduction; 25 kHz, 1.4 logs; 32–40 kHz, 1.3 logs; 62–70 kHz, 1.3 logs) had no effect on decontamination [68].

Ultrasound treatment was found to be effective at reducing the concentration of *Salmonella typhimurium* on chicken breast skin [69]. Decontamination treatments in a chlorine solution (0.5 ppm free residual chlorine) reduced *Salmonella typhimurium* contamination by 0.2 to 0.9 logs, while ultrasound treatment (20 kHz, 100 W) reduced counts by 1 to 1.5 logs. A combination 0.5% ppm chlorine and ultrasound treatment was the most effective, reducing *Salmonella typhimurium* by 2.4 to 3.9 logs.

Combined chemical (acetic acid) and ultrasound treatments (frequency not given; 240 W; up to 320 seconds) have also been used to clean egg shells [70]. The ultrasound and acetic acid treated eggs were as clean as commercially washed eggs, and ultrasound and acetic acid treated eggs had the same sensory properties and foaming ability, and gave cakes with the same height as eggs cleaned in acetic acid without ultrasound.

43.5.6 Decontamination of Processing Surfaces

Cleaning of surfaces used for food processing is also important, as they can be a cause of cross-contamination. Ultrasound treatment can improve the removal of microbial spores from surfaces [71, 72]. However, the effectiveness of ultrasound recovery is affected by factors including the material, treatment time, type of microorganism, and presence of surfactants [71–73]. The material can affect recovery substantially, with the recovery of fungal (*Aspergillus niger*) spores from PVC (50%), frosted glass (60%), and plain glass (80%) being higher than stainless steel (10%) or HDPE (10%) [72, 74]. There was similar variation in the recovery of bacterial

(*Bacillus subtilis*) spores, with high recovery from glass (96%), and lower recoveries from the plastics (polystyrene, 24%; polypropylene, 53%; polyethylene, 41%; polycarbonate, 46%) [71]. Addition of a surfactant (Tween 80) enhanced ultrasound recovery for polystyrene (42%) and polyethylene (75%), but had little effect on glass (92%), polypropylene (51%), and polycarbonate (43%).

Ultrasound treatment has also been shown to be effective at removing protein contamination from knives in abattoirs [75] and milk biofilms on stainless-steel and polypropylene dairy processing equipment [76]. A number of studies have demonstrated the use of ultrasound in cleaning cheese molds [63]. In a practical investigation in a commercial chicken processing plant, ultrasound-assisted cleaning of plastic trays, steel baskets, and steel shackles was effective at reducing microbial and other contamination [77].

43.6 ENHANCEMENT OF MASS TRANSFER

Mass transfer plays an important role in many industrial processes, such as drying, dewatering, filtration, membrane separation, salting, osmotic dehydration, extraction, sonocrystallization, and rehydration. Ultrasound has been shown to improve the efficiency of many mass transfer processes either through a direct involvement in the process (e.g. cavitation, microstreaming, or acoustic streaming causing agitation to improve mass transfer, cavitation creating voids in the material), or supporting the process (e.g. agitation or microjet formation to assist cleaning). There has been substantial research into ultrasound-assisted drying, membrane filtration, osmotic dehydration, and sonocrystallization along with the related processes of cheese brining, meat curing, and rehydration of dairy powders. There has been substantial research into industrial applications of ultrasound-assisted bed filtration and dewatering [78–82], where it has been shown that ultrasound can substantially improve the efficiency of dewatering or filtration systems.

43.6.1 Drying

Drying is a mass transfer process involving the removal of liquid from a material, with the rate of drying dependent on factors such as the structure of the material, temperature, relative humidity of the air, and air velocity. In selecting drying conditions it is necessary to find a balance between conditions that maximize the drying rate and minimizing any undesirable changes these conditions will cause in the product.

As a result of the effects of ultrasound on material properties, incorporating ultrasound into the drying process can increase the rate of drying by reducing external barriers causing agitation in the drying medium close to the solid surface which reduces the thickness of the boundary layer [83] and internal barriers by enhancing internal liquid movement from the core of the material to the surface due to repeated compression and expansion or the creation of micro-channels in the solid that permit liquid movement [83]. Combining ultrasound and air drying has been shown to enhance the rate of drying in various products including carrots slices [84], onion slices [85], potato cylinders [86], wheat [87], corn [87], rice [88], and walleye pollack surimi (washed fish mince) [89].

While ultrasound is normally used with solids or liquids, as they provide a good medium for the propagation of ultrasound, various systems have been developed that will generate ultrasound in gases, such as the Galton whistle or a siren [30], or a piezoelectric transducer with radiating plate. Stepped-plate radiating plate designs have been developed which improve the efficiency of ultrasound generation when compared to conventional flat plate designs [90].

The effectiveness of sound or ultrasound frequency and intensity tend to depend on the particular combination of material, drying conditions, and ultrasound system. For example, in some instances in onion rings, the drying rate increases with an increase in sound frequency from 1.6 to 3.2 kHz (both at 140 dB) [85], while for green rice there was little difference between 12 kHz and 19 kHz, though relatively low intensities were used (132 dB and 128 dB respectively) [88]. With potato cylinders dried with combined hot air (120°F) and sound (0.7 kHz to 10.25 kHz), a sound frequency of 8.1 kHz was found to be most effective [86]; however due to the experimental set-up used, the sound intensity decreased with increasing frequency (142 dB at 0.7 kHz; 102 dB at 10.25 kHz) making the results more difficult to interpret.

The greatest benefit of combining ultrasound and air drying occurs at lower temperatures. In carrot slices, a combination of ultrasound and 60°C air took about 25 minutes to reduce carrot slice weight by 80%, while 60°C air alone took about 35 minutes [84]. By contrast, combined ultrasound and 115°C air drying gave no benefit over drying in 115°C air alone. In walleye pollack surimi, a combined ultrasound and air drying rate reduced the time to dry from 73% moisture to 60% moisture at 20°C from 260 minutes (0 dB) to 70 minutes (155.5 dB), while at 50°C drying time reduced from 100 minutes (0 dB) to 40 minutes (155.5 dB) [89]. A similar pattern, where the benefit from ultrasound treatment (11.7 kHz; 165 dB) decreased with increasing drying temperature, was also seen in whole and crushed wheat and crushed corn [87].

Another application of ultrasound in drying has been to assist the drying of liquid droplets. Incorporating ultrasound in a spray drying tower reduced the maximum temperature of the material during drying and reduced the size of the dried particles [91]. Ultrasound has also been used to further reduce the size of droplets originally produced by spraying a liquid through a nozzle, reducing the drying time required [92].

43.6.2 Osmotic Dehydration

Osmotic dehydration is a food preservation technology that involves soaking food products (generally fruits and vegetables, though also with cheese and meat) in a concentrated solution (e.g. sodium chloride and sugar). In a relatively slow process, water moves from the product into the high-concentration solution, solutes move from the solution into the product and there is movement of solutes from the plant cells into the high-concentration solution [83, 93–95]. Osmotic

dehydration can improve the sugar-to-acid balance, texture, and color stability, and is typically used as a pre-treatment before freezing or drying [93].

Ultrasound treatment has proven to be an effective technique at increasing the rate of osmotic dehydration. When apple cubes were dehydrated in a 70°Brix sucrose solution, the rates of water loss and sucrose gain were higher than in samples agitated in an oscillating water bath [96]. Ultrasound treatment was also able to increase the rate of osmotic dehydration of strawberry halves [97]. In strawberries that had been pre-treated with high pressure, a combination of osmotic dehydration and ultrasound treatment (35 kHz water bath) increased the water loss and solids gain compared to osmotic dehydration alone, though a combination of osmotic dehydration and vacuum was the most effective treatment.

Not all products respond in the same way to ultrasound-assisted osmotic dehydration. Differences in water loss or solids gain in products treated with osmotic dehydration or a combination of ultrasound and osmotic dehydration has been attributed to differences in the structure of the food product [83].

43.6.3 Cheese Brining

The manufacture of some cheeses and cured meats relies on the penetration of brine into the food product, as the salt alters the flavor and texture development, reduces water content, improves microbial safety, and contributes to the maturation of the product [98]. When brining is used with larger items, the process can be time-consuming as mass transfer can be relatively slow. Various methods can be used to improve the rate of brine penetration, such as agitation or tumbling, vacuum, increased brine concentration, increased brining temperature, ratio of brine to product, and needle injection. However, it is important to maintain finished product quality. For example, injection of brine directly into meat using multi-needle injectors is common in meat processing, but the path where the needle penetrated is sometimes obvious in the cooked product.

Ultrasound can improve the brining process in cheese, increasing the rate of water loss and sodium chloride gain from small cheese blocks (cylinders 34 mm in diameter and 30 mm high; parallelepipeds of 60 mm by 25 mm by 12.5 mm) [98]. The benefit of ultrasound occurred over a range of temperatures (5 to 20°C), and was noticeable when compared to static or agitated brining. In larger samples (parallelepipeds of 140 mm by 140 mm by 90 mm), ultrasound treatment increased the initial sodium chloride penetration particularly at the surface [99]. However, the moisture content diffusion rate was lower in ultrasound-treated cheese than conventionally treated cheese, and both ultrasound and conventionally brined cheeses reached a consistent sodium chloride distribution after the same maturation time [99]. The use of ultrasound brining can affect other characteristics, with more rapid formation of free amino acids [100] and free fatty acids [101] during the maturation of acoustically brined cheeses, and differences in the sensory texture, aroma, odor, and taste between ultrasound and conventionally brined cheeses [101], though no differences were found in cheese microstructure [99].

43.6.4 Curing Meat Products

The functional properties of many processed meats rely on the partial solubilization of muscle protein in the raw meat (generally by sodium chloride or a combination of sodium chloride and polyphosphate) in order to increase the cook yield and to hold meat pieces together after cooking. One common approach used to make restructured meat products containing large pieces of meat is to tumble (mix) the meat chunks with the salts (added either as dry salts, or dissolved to form a brine), relying on the mechanical action to distribute the salts through the meat, and to solubilize part of the meat protein. The tumbling process is time-consuming, requiring several hours, and ultrasound has been used to reduce or eliminate the tumbling process.

A combination of treatment with ultrasound and tumbling gives products with equal or higher bind strengths and cook yields than meat tumbled without ultrasound [102, 103]. The increase produced by the combined ultrasound and tumbling treatment varies, with the largest increases occurring in meat with no added salt, with the effectiveness of ultrasound progressively decreasing [102] or disappearing [103] with increasing level of salt addition. Ultrasound also proved effective in meat when used without any tumbling. In meat injected with a brine, either a conventional tumbling process or ultrasound treatment (22 kHz) was used to distribute the brine through the meat [104]. The ultrasound-treated meat was more tender and juicier, with a higher production yield than tumbled meat. In all studies, ultrasound treatment affected the microstructure of the treatment, with greater separation of myofibrils occurring in the ultrasound only [105], or combined ultrasound and tumbling samples [102, 103] than in the tumbling-only samples.

43.6.5 Membrane Filtration

Membrane separation techniques, such as microfiltration, ultrafiltration, and reverse osmosis, are widespread in food processing to concentrate or purify materials for the dairy, beverage, and egg industries [106–108]. A disadvantage of membrane separation techniques is that they show a progressive decrease in flow rate due to fouling and concentration polarization. Fouling is a result of components from the feed being deposited on the surface or pores of the membrane, while concentration polarization occurs due to concentration gradients that develop due to the accumulation of retained components near the membrane [109]. Membrane systems are operated to minimize these problems so that they maintain their flow rate over the course of a day's processing (e.g. periodic flushing to remove fouling, feed flow velocity and turbulence to minimize concentration polarization) [108], and many strategies are employed to clean membrane systems [110]. Ultrasound can be used to improve the efficiency of both the membrane filtration process [111], and cleaning of fouled membranes [109]. Ultrasound-assisted membrane processing has been used successfully with a variety of food-related systems, including solutions of salt, polysaccharides and proteins, and yeast cell suspensions.

Membrane distillation is a process involving the membrane separation of a vapor and a liquid, for example in desalination or concentration of solutions. Ultrasound increased permeate flux during membrane distillation by 5% to 30% compared to the conventional process [112, 113]. In addition to ultrasound's effect on concentration polarization and fouling found in other membrane separations, combining ultrasound with membrane processing also reduces temperature polarization (temperature gradient between bulk liquid and membrane).

The filtration of suspended solids can also be enhanced by ultrasound treatment. There was a higher flux when ultrasound was used in conjunction with microfiltration to dewater yeast cells suspended in water [114]. The benefit of ultrasound decreased as the liquid feed velocity increased, and at 0.53 m/s there was no additional increase in flux due to ultrasound [114]. Ultrasound treatment improved permeate flux during the microfiltration of baker's yeast suspensions with either continuous pump pressure and ultrasound, or intermittent pump pressure and ultrasound, and was also effective at cleaning a membrane that had been fouled during ultrasound processing [115].

The use of ultrasound in preventing or removing fouling of membranes [109] depends on ultrasound parameters (e.g. ultrasound frequency and intensity), the filtration system (e.g. intermittent or continuous ultrasound application during filtration, conditions during cleaning cycle, acoustic properties of the filtration membrane), attenuation of the ultrasound signal by the feed material, size of colloidal particles in the feed material, and the cavitation threshold of the feed material (viscosity, temperature, surface tension, pressure, dissolved gases, concentration). Ultrasound treatment does not affect membrane permeability, rather it helps prevent the decrease in permeate flow rate by breaking up fouling at the membrane surface or reducing the concentration polarization, though it may be less effective at removing material stuck in membrane pores [109].

One limitation of ultrasound in membrane processing is the potential for damage to the membrane through processes such as cavitation-induced erosion, acoustic streaming, and microstreaming. Some studies have shown that ultrasound affects membranes altering both permeability and microstructure, while others have found no effect [109]. This may be due to differences in susceptibility to ultrasound depending on membrane material [116, 117] and ultrasound parameters [117].

43.6.6 Extraction

Extraction is used to separate compounds out of their surrounding matrix. Utilization of ultrasound in extraction has emerged in the food industries due to its benefits in providing comparable effects to other novel extraction technologies (supercritical fluid extraction, high pressure processing, microwave-assisted extraction, and pulsed electric field) [118–122] and minimizing use of harmful solvents (i.e. chloroform, chlorobenzene, methanol, and acetonitrile) compared to the conventional solid–liquid extraction methods [123, 124]. Ultrasound-assisted extraction (UAE) used with non-toxic solvents such as water, ethanol, and sunflower oil has been investigated in a variety of food matrices to extract a range of compounds including carotenoids, phenolic compounds [125], natural pigments [126], oligosaccharides [127], polyphenols [128, 129], and anthocyanins [130].

Upon sonication, ultrasonic cavitation generates massive changes in temperature (2000–10,000 K) and pressure (100–1000 MPa) in the medium, leading to mechanical and cavitational effects on the extraction process. In the first stage, there is shear disruption that causes damage to the cellular matrix and particle size reduction [131]. Physical modification of the matrix surface creates pores that improve solvent penetration into the internal structure by mechanical effects, facilitating the disintegration of intracellular materials. Ultrasonic cavitation also induces the formation of microfissures and microchannels in the matrix surface that further enhances the penetration of the solvent into the matrix. In the second stage, an increase in surface area of the solvent–matrix interface allows a greater dissolution of extracting compounds to the solvent [132]. The mass transfer and diffusion of the target compounds into the solvent are also facilitated by turbulence mixing and acoustic streaming [133].

Process efficiency, extraction yields, and quality of extracting materials using the ultrasound method depend on various factors such as sonication parameter (ultrasonic power and frequency), extraction parameter (extraction time and temperature), solvent properties (polarity, viscosity, vapor pressure, surface tension, etc.), particle size of matrix, the ratio of solvent to matrix, and the interaction between solvent and matrix [134, 135]. For example, when extracting prebiotic oligosaccharides from selected fruits and vegetables using ultrasonic water bath at 40 kHz, the yield of total oligosaccharides increased two- to four-fold compared to that extracted by conventional methods. The optimum yield of total oligosaccharides can be achieved with a sonication temperature of 40°C, sonication time of 10 min, and ethanol concentration of 63% v/v [127]. A previous study performed on the extraction of phenolic compounds from grape pomace reported that the UAE method surpassed the conventional aqueous extraction method with a reduction of extracting time by three- to eight-fold depending on the sonicating temperature (20–50°C) [136]. Attempts have also been made to employ UAE in a solvent-free extraction system using vegetable oils as alternatives to organic solvents for extracting carotenoids in carrot and pomegranate wastes [137, 138]. These studies demonstrated that sonication and the use of vegetable oils improved extraction yield and reduced extraction time [138]. UAE can be combined with supercritical fluid extraction [139], microwave [140], high pressure [141], and enzymes [142, 143] to provide cumulative effects in extraction process.

43.6.7 Sonocrystallization

43.6.7.1 Ultrasound Treatment Alone

The crystallization of food systems can be categorized into supercooled and supersaturated crystallization [144]. Typically, the former involves freezing of food where water

transforms into ice crystals whose size, shape, and distribution can be modulated by the ultrasound technique. The application of ultrasound in the freezing of food is discussed in Section 43.6.1. Supersaturated crystallization is the main mechanism to induce condensation of solute from saturated solution, which plays a pivotal role in the crystallization of sugar, fat, and protein [144]. Conventional supersaturated crystallization (addition of seed crystals and use of an antisolvent) is reported to be time-consuming whereas the use of power ultrasound to assist crystallization or "sonocrystallization" is known to increase nucleation rate in the primary nucleation, induce fragmentation of crystals in the secondary nucleation, and facilitate crystal growth rate [145–149]. These advantages of sonocrystallization are based on the effect of acoustic cavitation bubbles acting as nuclei that accelerate the induction of nucleation and subsequent crystal growth. In addition, the cavitation effect also enhances movement of solutes and subsequent collisions of particles [150]. These effects lead to changes in supersaturation and reduce the metastable zone width whereby a controlled crystallization process can be achieved. The crystal habit (size, shape, and distribution) and product yield obtained by ultrasonic method are attributed to sonication conditions (frequency, power, intensity, pulse mode, and time) and levels of supersaturation (concentration and crystallization temperature) [151–154].

43.6.7.1.1 Lactose

Sonocrystallization of lactose resulted in the formation of smaller size, narrow size distribution [155–157], higher lactose recovery, and higher purity [153, 158, 159].

43.6.7.1.2 Edible Fats/Oils

High-intensity ultrasound (acoustic frequency 20 kHz, electrical power 50 W, and sonication time 10 s) also modified the crystallization behavior of edible fats, such as palm kernel oil, shortenings, and anhydrous milk fat. A decrease in induction time and a generation of smaller fat crystals influenced the polymorphic forms, melting behavior, microstructure, and texture of those fat samples [152].

43.6.7.1.3 Food Protein

When ultrasound (100 kHz and 100 W) was applied to an egg albumin solution, it was found that a sonication time of 10 s increased the nucleation rate whilst longer-term sonication tended to damage the crystal cluster due to excessive irradiation [160].

Apart from lactose, anhydrous milk fat, and egg albumin, the application of ultrasound to crystallization can also be found in other food products such as triglycerides, cocoa butter [161], soybean oil [162], and palm oil [163].

43.6.7.2 Ultrasound Treatment in the Presence of Dissolved Gases

The presence of dissolved gases (e.g. air, oxygen, carbon dioxide) in the food systems has a significant impact on ultrasound treatment, leading to changes in crystal size, number, and distribution. The presence and motion of gaseous bubbles themselves are capable of providing nucleation sites. In addition, any mechanical treatments (i.e. mixing, shaking, sonication, etc.) applied to the system can create nano- to micron-sized gaseous bubbles from the dissolved gas. The tiny gaseous bubbles can act as catalysts under sonication, causing a synergetic effect on crystallization behavior. As a result, the number of nuclei and resultant crystals is greatly increased whereby the physical properties of sonicated foods can be modified.

It has been reported that coupling dissolved oxygen and ultrasound (35 kHz, 1 s, 0.2 W/cm^2) in mannitol solution (10%wt) resulted in a substantial decrease in crystal size whereas uniformity in size of the single crystals was enhanced [164]. Recent studies performed on lactose and anhydrous milk fat have demonstrated that the addition of dissolved carbon dioxide at a concentration of 2000 ppm prior to the application of ultrasound (20 kHz, 1 min) could enhance the effects of sonication on facilitating the initiation of nucleation and the reduction of crystal size. This leads to an increase in lactose recovery with smaller crystal size compared to using ultrasound alone [157].

For lipid systems, the addition of carbon dioxide in sonicated materials can be beneficial for minimizing the lipid oxidation during storage which is accelerated by the presence of hydroxyl and hydrogen free radicals formed during the sonication of fats and oils. A similar effect has also been reported in sonication of carbon dioxide-infused gelatin gel at low frequency (20 kHz, intensity of 0.7 W/cm^2). The combined treatment caused a decrease in freezing time, ice crystal size of the gels, and water loss [165].

43.6.8 Rehydration of Dairy Powders

In order to utilize reconstituted dairy powders for food processing, it is important that they are completely dispersed and dissolved into solution. Thus the solubility of the reconstituted dairy powders is a key influential factor on functionality and product performance. Failure to rapidly achieve complete rehydration will cause economic losses due to time-consuming rehydration, processing difficulties, and decreased productivity [166].

Numerous studies have shown that reconstituted dairy powders enriched with protein and casein-dominant items such as milk protein concentrates (MPC) are prone to poor solubilization [167–169]. The poor solubility of MPC is not fully understood but is assumed to be induced by the formation of a network of crosslinked proteins on the surface of powder particles [170]. The rehydration of dairy powders involves two dissolution steps in which the disruption of primary particles occurs in parallel with the release of materials into aqueous phase [170]. The use of ultrasound is an effective physical approach to increase the dispersibility of poorly soluble milk powders as, owing to its generated cavitation effects, it can break the insoluble agglomerated masses into smaller particles, facilitating the simultaneous discharge of materials into the surrounding medium [171].

It was demonstrated on whey protein powders that low frequency (i.e. 20 kHz) surpasses high frequency in terms of

physical size reduction of primary particles. A considerable reduction in whey protein powder from 30 μm to 1 μm was achieved for the first 10 min of sonication at 20 kHz and 31 W [172]. Kresic and Lelas [173] also reported that sonication treatment at 20 kHz for 15 min increased the solubility of whey protein isolate up to 98.9%. McCarthy and Kelly [174] observed that ultrasonication at 20 kHz for a very short time period (0.5 min; energy density 10.5 J mL^{-1}) was sufficient to dissociate MPC powder particles into smaller ones, yielding 95.6% MPC solubility.

Low-frequency ultrasound also induces complete rehydration of MPC powders after 10 min of ultrasonication whereas mechanical stirring treatment took about 4 h to achieve a comparable solubility (85%) [175]. In contrast, high-frequency ultrasound (213–644 kHz) did not induce any size reduction of whey protein powders at 8% w/w solid concentration [172, 176]. A previous study performed on ultrafiltered and diafiltered milk protein retentates showed that ultrasonication can be used as an additional step prior to spray drying to improve the solubilization of dairy powders. When the milk protein retentates were sonicated at 20 kHz and 12.5 W for 0.5 min, the particle size decreased from 28.45 to 0.13 μm. The sonicated milk protein retentates were subjected to spray drying. The solubility of the resultant powder remarkably increased from 35.78 to 88.30% [177].

43.7 ENHANCEMENT OF HEAT TRANSFER

Sound and ultrasound can be used to enhance the rate of heat transfer during freezing [178-–181], thawing [182], and cooking [183, 184]. In the case of thawing and cooking, sound and ultrasound assist the process by increasing the rate of heat transfer with the surrounding medium and by absorption of sonic energy by the material. By contrast, in samples being frozen, the absorption of energy from sound waves which will reduce the cooling rate needs to be balanced against the improved heat transfer coefficient and also improved product quality produced by increasing ice crystal nucleation.

43.7.1 Freezing

Ultrasound can assist in the freezing process, both through decreasing the time required to freeze the food, and improving the quality of the frozen food [180]. Ultrasound can be used to increase the convective heat transfer coefficient [180, 185, 186] between the food being frozen and the refrigerant. Ultrasound also affects crystallization, increasing the nucleation rate and crystal growth rate [35]. As the freezing of water in food is a crystallization process, ultrasound can alter this process, possibly through cavitation bubbles being nuclei for ice crystal formation, or through collapsing cavitation bubbles shattering existing ice crystals creating more nuclei [179, 180]. Since reducing the size of ice crystals in frozen food helps to reduce damage to the food microstructure and improve the quality of the food when consumed, using ultrasound to assist freezing may improve the freezing rate and quality of the frozen product.

A disadvantage of using ultrasound to improve the freezing process is that ultrasound also generates heat, so the benefits of improved heat transfer and nucleation must be balanced against heating. In a study of freezing potato pieces ($76 \times 17 \times 17$ mm^3) in an ultrasonic bath (25 kHz), a power input of 7.3 W did not substantially affect the freezing rate (compared to no ultrasound), while 15.9 W and 25.9 W both decreased the time to go from 0 to −7°C, though 15.9 W gave a greater reduction in 0 to −7°C time than 25.9 W [179]. The limited effectiveness of 7.3 W was ascribed to the low intensity of agitation produced causing little increase in heat transfer. The greater effect on the cooling rate at 15.9 W than 25.9 W was attributed to the balance between the improved heat transfer between the refrigeration medium and the potato due to greater agitation, and the higher heat production due to greater energy input by the more intense ultrasound [179].

Selecting the appropriate ultrasound intensity also affected the microstructure of frozen potato [178]. The ultrasound treatment found to give the best freezing rate (15.9 W) was also found to minimize microstructural change. Immersion freezing combined with a 15.9 W ultrasound treatment gave tightly packed cells, with no observed cell wall damage, compared to no-ultrasound immersion freezing which caused cell wall damage and caused separation of the potato cells. Compared to the other ultrasound treatments, 15.9 W caused less damage than 7.3 W ultrasound treatment (prominent cell wall separation and intercellular voids), with a 25.9 W ultrasound treatment causing an intermediate level of damage compared to the 7.3 W and 15.9 W treatments [178].

43.7.2 Thawing

Large-scale thawing of frozen food is traditionally done with air or water thawing, though there is interest in emerging technologies such as high-pressure, microwave, ohmic, and acoustic or ultrasonic thawing [182]. Thawing techniques should minimize the thawing time, and minimize the formation of hot spots in the food that can damage quality (e.g. partial cooking, or increased microbial growth) while maintaining product quality.

Early attempts at ultrasound or acoustic thawing were not particularly successful, with the formation of hot spots, poor penetration, and high power consumption [187]. More recently, an improved understanding of the interaction between sound waves and food products has improved the results of acoustic or ultrasound thawing, including using ultrasound either to assist thawing [188], or as the only energy source for thawing [189].

It has been proposed that the absorption of ultrasound energy depends on the thermoelastic relaxation of ice crystals in the food, and is affected by ice crystal orientation and size, impurities present in the ice crystals, and temperature [188]. Studies in meat found differences in ultrasound attenuation depending on food temperature, with greater attenuation in frozen meat than thawed [190], and increasing attenuation as the temperature of frozen meat increased markedly above about −10°C reaching a maximum near its freezing point (about −2°C) before decreasing rapidly at higher temperatures

[191], making ultrasound particularly suitable for controllable thawing of foods [189].

Effective acoustic or ultrasound thawing relies on the selection of an appropriate frequency and intensity to defrost the food efficiently without excessive heating near the surface [189]. When ultrasound was applied directly to meat (beef, pork, or fish) excessive heating near the surface was particularly a problem at high intensities (1 to 3 W/cm^2) and over a range of frequencies (220 kHz to 3.3 MHz), with cavitation causing problems at lower frequencies, while high attenuation caused excessive heating at higher frequencies [189]. A combination of 500 kHz and 0.5 W/cm^2 was found to offer effective thawing while minimizing surface heating.

Acoustic defrosting (1500 Hz) of fish blocks in an agitated water bath (18°C, 3.8 m/s water velocity at block surface) reduced the time to go from –29 to –1°C by approximately 25 to 70% (depending on ultrasound power input level), while larger reductions were seen going from –5 to –1°C (approximately 32 to 82%) [188]. Some rapid thawing techniques can cause excessive heating at the product surface leading to loss of product quality. However, combined acoustic and water bath thawing gave the same surface temperature as water bath thawing alone [188].

43.7.3 Cooking

Some use of ultrasound has been made for cooking of meat, taking advantage of the increase in temperature due to the absorption of the ultrasound energy. Several ultrasound cooking processes have also been developed for meat products [192, 193], and similar processes may be useful with other foods. Ultrasound cooking (1000 W, 20 kHz) of beef samples in a water bath reduced the cooking time (6.7 minutes), reduced energy consumption (0.08 W/g), and gave a higher yield (85.3%) than convection cooking in a broiler (12.3 minutes; 0.22 W/g; 76.1%) [184]. Ultrasound cooking gave similar or better tenderness ratings than convection cooking but had a poorer flavor. An important disadvantage of substituting ultrasound cooking for other methods is the poorer flavor [184]. The flavors formed during dry heat cooking of meat (e.g. roasting, grilling) are an important part of the sensory characteristics, with different volatiles profiles forming during moist cooking (e.g. boiling, microwave) [194, 195].

In addition to using ultrasound in isolation to cook food, ultrasound-assisted water bath cooking is also likely to prove effective, due to the increased heat transfer coefficient produced by ultrasound [185, 186], and the agitation of the liquid by ultrasound mixing helping to ensure an even temperature throughout the cooking medium [184].

43.8 PROCESSING OF PROTEIN FOODS

Ultrasound can be used to enhance the processing of materials containing proteins, such as the tenderization of raw meat, destruction of microorganisms, homogenization, protein extraction from cells, and enhancing membrane filtration. The ultrasound mechanisms that can enhance these processes, such as localized or bulk heating, micro-jet formation, high shear, liquid agitation, polymer chain lysis, and free radical formation, can also denature the proteins present. In some instances, this can be desirable, such as the denaturation of proteins responsible for undesirable texture, color, or flavor changes, while in other situations it can be undesirable, for example giving a cooked appearance to raw meat during tenderization or loss of activity in enzymes being extracted from cells and purified. As a consequence, each process must be developed to maximize or minimize the effect of ultrasound on the proteins present.

43.8.1 Enzyme Inactivation

Ultrasound increases the effectiveness of heat inactivation of enzymes, and has been demonstrated in enzymes derived from plants [soybean lipoxygenase [196]; horseradish peroxidase [196–198]; watercress peroxidase [199]; mushroom polyphenol oxidase [196]; orange pectinmethylesterase [200]; tomato pectinmethylesterase and polygalacturonase [201]]; animal tissues [porcine heart malate dehydrogenase [202]; rabbit muscle L-lactic dehydrogenase [202]; bovine intestinal mucosa alkaline phosphatase [202]; porcine pancreas phospholipase A2, trypsin, and lipase [203]; bovine pancreas α-chymotrypsin [203]; trypsin, [204]], microorganisms [*Pseudomonas fluorescens* lipase and protease [205]; alcohol dehydrogenase and glucose-6-phosphate dehydrogenase from baker's yeast [202]; β-galactosidase from *Escherichia coli* [202]]; and milk [bovine lactoperoxidaase and alkaline phosphatise [206]; alkaline phosphatase, γ-glutamyltranspeptidase, and lactoperoxidase [207]]. The ultrasound stability of individual proteins varies between enzymes [196, 202, 203, 205] and also depends on ultrasound treatment conditions [196, 198, 202, 204, 205, 208], the composition of the treatment medium [196, 198, 200, 202–204, 209], treatment pH [205], and whether they are bound (e.g. membrane-bound proteins) or free (e.g. cytoplasmic proteins). Enzyme inactivation generally increases with increasing ultrasound power, ultrasound frequency, exposure time, amplitude, cavitation intensity, processing temperature, and processing pressure, but decreases as the volume being treated increases [196–200, 202, 204–206, 208].

A combined heat and ultrasound treatment can produce a markedly greater effect on enzyme inactivation than heat alone. Orange pectinmethylesterase in orange juice was inactivated relatively slowly by heat alone (72°C, D value of 500 minutes), while the combined heat and ultrasound treatment (72°C, 20 kHz, 117 μm, 350 kPa) gave a much lower D value (1.2 minutes) [200]. Ultrasound was also effective at a temperature (38°C) where thermal inactivation is insignificant, giving a pectinmethylesterase D value of 11 minutes. The rate of inactivation of tomato pectinmethylesterase was also greatly increased by a combination of heat and ultrasound, with increasing cavitation intensity (measured via H_2O_2 production) dramatically increasing the rate of inactivation [208]. Similarly for *Pseudomonas fluorescens* heat-resistant lipase, a combined ultrasound and thermal treatment (110 to 140°C, 20

kHz, 117 μm, 350 kPa) reduced lipase D values by 25 to 86% (average of 58%) and protease D values by 15 to 67% (average of 42%) compared to the heat-only treatment at the same temperature [205]. Generally, the reduction in D value tended to decrease with increasing treatment temperature. In milk, ultrasound alone is not very effective at inactivating milk enzymes (alkaline phosphatase, γ-glutamyltranspeptidase, and lactoperoxidase) and other milk proteins (α-lactalbumin and β-lactoglobulin), but a combination of ultrasound and heat is more effective than heat treatment alone [207].

Studies with buffers show that making small changes in the treatment medium composition, such as adding calcium salts, or whey protein hydrolysate, can markedly alter the rate at which an ultrasound treatment denatures enzymes [203]. For example, in a salt buffer adding calcium increased the stability of α-chymotrypsin by 62%, while in a whey protein and salt buffer, adding calcium increased the D value by 146% [203]. There was a marked difference between the salt buffer and the whey protein and salt buffer (511% no calcium, 826% with calcium). By contrast, there was little difference in trypsin inactivation between the salt buffer or whey protein buffer either with or without added calcium.

43.8.2 Changes in Protein

Ultrasound-induced changes in milk also affect the characteristics of products made from the milk. Yogurt manufactured from milk processed with ultrasound (40°C, 20 kHz, 2 kg/cm^2, 12 s ultrasound exposure time) took 10 to 20% longer to ferment (slower pH fall), lost less serum, and had a different texture (higher compression force, higher viscosity) than yogurt made from conventionally processed milk [205]. These differences may be due to ultrasound-induced protein modification. In another study, milk treated with ultrasound (20 kHz, 15 to 20°C, 6 minutes ultrasound exposure time) before a yogurt starter culture was added fermented at effectively the same rate (indicated by pH fall) as a conventionally homogenized milk [210]. In contrast, fermentation was faster when samples were treated with ultrasound during fermentation, which may have been due to faster lactose hydrolysis as a result of ultrasound causing the release of β-galactosidase from bacterial cells [211]. Some ultrasound treatments also increased yogurt viscosity, increased water-holding capacity, and reduced syneresis compared to yogurt made with conventionally homogenized milk, which are likely to be the result of changes in protein conformation caused by ultrasound. Some depolymerization of collagen is also reported [212].

43.9 ULTRASOUND AS A PROCESSING AID

Ultrasound has been used to enhance the efficiency of food-related enzymatic processes including enhancing the esterification of glucose [213], hydrolysis of olive oil [214], and proteolysis of casein by α-chymotrypsin [215]. Ultrasound increased the rate of invertase catalyzed hydrolysis of sucrose, α-amylase and glucoamylase hydrolysis of starch, and α-amylase hydrolysis of glycogen [216, 217]. These studies show that ultrasound power levels can be found that give a balance between enhanced rates of enzyme activity while maintaining enzyme activity over an extended period. Ultrasound has also proven effective when lactose in milk is hydrolyzed by fermentation with *Lactobacillus bulgaricus* [211]. Ultrasound increased the rate and extent of lactose hydrolysis, and also altered the hydrolysis process, giving markedly higher levels of residual glucose in the milk. When lactose is hydrolyzed within the cell, the glucose produced is consumed by the bacteria.

43.10 HOMOGENIZATION AND EMULSIFICATION

Homogenization or emulsification is one of the important unit operations in the food industries. Homogenization is a common term used when the size of a non-uniform dispersed system is reduced to a required size and distributed uniformly in the bulk product. In the homogenized product, the particle size distribution of the dispersed phase is narrow. Milk is the food that is most commonly homogenized. In the emulsification process, two immiscible components are mixed and uniformly distributed. The examples are oil-in-water or water-in-oil type emulsions. The equipment used to homogenize or emulsify the product are high-pressure homogenizers and colloid mills. Ultrahigh-pressure homogenizers (can go up to 5000 bars) and microfluidizers are recent techniques that are gaining popularity in the preparation of sub-micron emulsions. Ultrasonication has also been proven to be another way of mixing two immiscible liquids, and has been used successfully in the cosmetic, pharmaceuticals, chemical, and textile industries [131].

Ultrasonic emulsification is mainly driven by cavitation, wherein the bubbles collapse at the interface of two immiscible continuous and dispersed phases. A straightforward way to produce an emulsion by ultrasound is by immersion of a sonotrode either into the mixture of all components or into the continuous phase and adding gradually the phase to be dispersed during sonication. This procedure works well for small batches, but scale-up is difficult [218]. Since the intensity of ultrasound in a liquid decreases rapidly with the distance to the sonotrode, it may be difficult to process larger volumes [218]**.** In some cases, the liquid whistle type of ultrasound device is used [131]. This can give much more throughput (up to 12 000 L/h) than the sonotrodes such as in the case of manufacturing fruit juices, ketchups, and mayonnaise [131]. Ultrasound treatment is also an effective method for homogenizing the fat globules present in milk [207, 210, 219]. Wu and Hulbert [210] reported that high-amplitude homogenization not only gave a good homogenization effect in milk but also significantly improved the water-holding capacity and viscosity and also reduced syneresis of yogurt produced from ultrasonicated milk. The ultrasound was also very effective in producing sub-micron sized emulsion of essential oil (limonene) for the purpose of encapsulation by spray drying. The particle size and distribution were the function of ultrasonication period (Figure 43.3). Ultrasonication was found

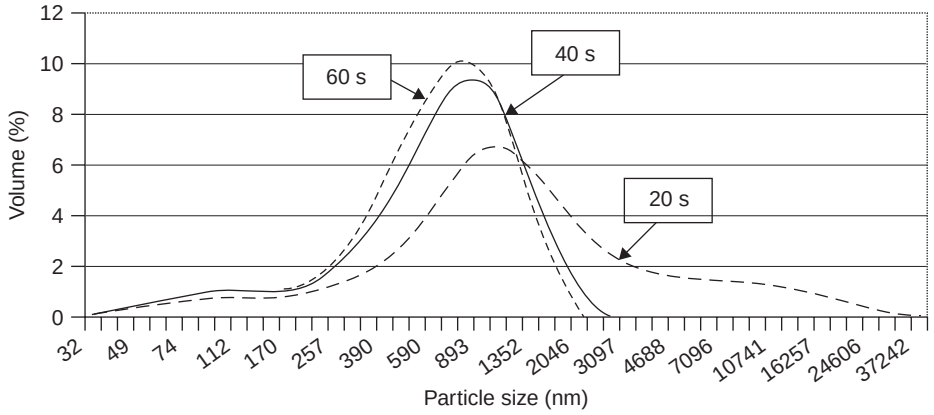

FIGURE 43.3 Size distribution of the emulsion produced by ultrasound at various time periods (dispersed phase-limonene, continuous phase Hi-Cap/maltodextrin mixture aqueous solution). (From Jafari et al. [220].)

much easier to operate, control, and clean as compared to the microfluidizer.

One of the issues of ultrasound emulsion is the critical product contamination of the metal alloys which are used to make the sonotrodes. This contamination can result in oxidation of the product and the development of off-flavor. Attempts have been made to make the device transmit the ultrasonic waves through other materials such as a double jacket filled with pressurized water in a flow-through cell where there will not be direct contact between the sonotrode and product [220].

43.11 MEAT TENDERIZATION

Tenderness is the most important consumer quality attribute in cooked meat [221, 222]. Meat tenderness is influenced by a multitude of factors, including animal characteristics (e.g. genetics, animal age, muscle function), pre-slaughter handling of the animal (e.g. stress, muscle glycogen), and post-slaughter handling of the carcass (e.g. chilling rate, electrical stimulation, carcass suspension technique) [223]. There are myriad techniques available to tenderize meat including many conventional approaches (e.g. aging time, treatment with proteases, injection with salt solutions, mechanical treatment, cooking technique), including some emerging techniques, such as hydrodyne (explosive shock), ultra-high pressure, and injection of calcium salts [223]. Ultrasound also offers potential as a tenderizing treatment in meat as it can be applied without causing the changes in appearance that occur with some other treatments.

The ultrasound conditions used to treat meat vary widely, and correspondingly the effectiveness of ultrasound for meat tenderization varies (Table 43.3). The intensity of ultrasound treatment appears to play an important role in the tenderizing effect, with studies using relatively low intensity (<2 W/cm^2) producing little effect, even though the samples received extended exposure (up to 70 minutes) [224, 225]. Higher-intensity ultrasound proved effective in some cases at tenderizing meat [104, 105, 183, 226–228], though a number of other studies found the treatment conditions to have no effect on tenderness [183, 184, 229, 230] or even to decrease tenderness [183]. The frequency chosen does not appear to be responsible for the variation in results, as these studies have been performed in a narrow range (20 to 47 kHz; most studies between 20 and 30 kHz). The use of high-frequency ultrasound (2.4 MHz) to treat pre-rigor meat initially increased the firmness (compression force) of raw meat compared to untreated or post-rigor ultrasound-treated meat [231]. After 14 days' aging, no difference was found between untreated, pre-rigor, and post-rigor ultrasound-treated meat [231].

The microstructure of ultrasound-treated meat shows that there can be significant disruption to the structure. In beef *Longissimus*, there was greater sarcomere shattering and myofibril disruption from ultrasound cooking than convection, though in the *Pectoralis*, no difference with cooking method was found [184]. This may be due to the greater collagen concentration and differences in connective tissue distribution in *Pectoralis* compared to *Longissimus*. In another study, both *Semimembranosus* and *Longissimus* showed damage to their microstructure caused by ultrasound [228] (Figure 43.4). By contrast, in chicken *Pectoralis* ultrasound treatment did not affect microstructure [227]. There was also greater myofibril damage in ultrasound-treated cured meat products [102, 105].

In beef *Semimembranosus* treated with high-frequency ultrasound (2.4 MHz), pre-rigor ultrasound-treated meat initially had longer sarcomeres than untreated meat, though there was no difference in myofibril fragmentation after 6 days' aging [231]. There were no differences in microstructure between post-rigor ultrasound-treated and untreated samples.

In addition to the physical disruption shown by microstructural studies, ultrasound could increase meat tenderness through an increase in proteolysis, which contributes to meat tenderization during aging [232, 233], for example through increased release of cathepsin (muscle protease) [233]. Ultrasound treatment of lamb muscle fibers was found to activate proteolysis, with SDS-PAGE analysis showing greater appearance of bands around 30 and 83 kDa, and the disappearance of an 87kDa band, in samples that had been ultrasound-treated and aged at 4°C for two days, compared to the much smaller changes in samples that had only been aged at 4°C for two days [234]. However, this effect is not found consistently,

TABLE 43.3
Some of the Studies of the Tenderization of Meat by Ultrasound Treatment

Muscle	Frequency (kHz)	Power Input	Treatment Time (min)	Tenderness Effect	Reference
Horse *Semimembranosus*	22	1500 W	Five sessions totaling 50 minutes	Ultrasound-treated meat had lower shear force (25.2 kg/cm^2) than conventionally processed (injected and tumbled) meat (31.5 kg/cm^2 N)	[104, 105]
Chicken *Pectoralis major*	40	2400 W	15	At 24 hours post-mortem, ultrasound-treated muscles gave a lower shear force (4.4 N) than untreated muscles (5.0 N)	[227]
Beef *Semitendinosus*	25.9	1000 W	Up to 16	Ultrasound-treated meat had lower shear force than untreated at 2 and 4 minutes, but equal or higher after 8 or 16 minutes	[226]
Beef *Longissimus Semitendinosus Biceps femoris*	30–47	0.29–0.62 W/cm^2	Up to 30	Ultrasound did not affect instrumental tenderness	[224]
Lamb *Longissimus*	20	63 W/cm^2	Average of 2.5 per steak	Ultrasound did not affect instrumental or sensory tenderness	[229]

as when beef or lamb muscles were treated with ultrasound, there was no effect on the SDS-PAGE profile, with the appearance of a 30 kDa band (a marker of proteolysis in aged meat) occurring at the same rate in ultrasound-treated and untreated meat [229, 230]. Ultrasound could also damage cell walls and membranes [13], physically disrupt proteins through bubble pulsation, cavitation, and free radical formation [13], including fragmentation of collagen [235]. An effect on collagen in treated meat has been shown, with meat cooked by ultrasound having a lower total and soluble collagen content than

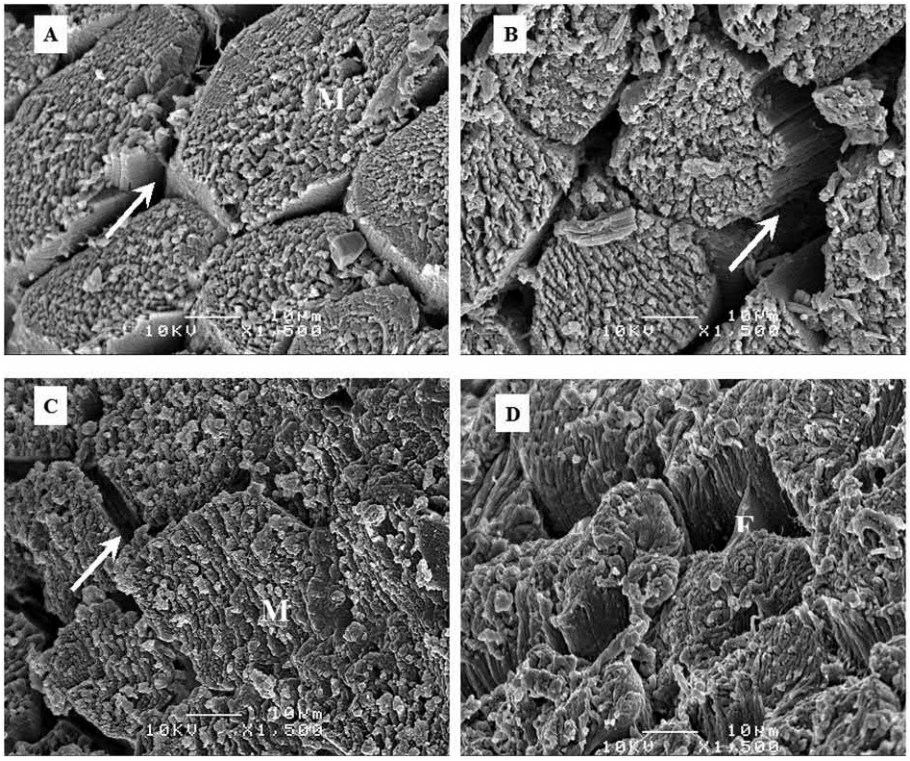

FIGURE 43.4 Scanning electron micrograph of bovine *Semitendinosus* muscle treated with ultrasound (24 kHz) for various treatment times. (From Jayasooriya [236].) (A) 0 s; tightly packaged and intact fiber bundles are shown, (B) 60 s; fiber bundles are intact, but increased spaces between fiber bundles are shown, (C) 120 s; disintegrated fiber bundles are evident, and (D) 240 s; integrity of the fiber bundles are lost, and myofibrils and connective tissue appeared to be denatured. M = fiber bundle; ⟶ = inter-myofibrillar spaces; E = endomysium. (From Jafari et al. [220].)

convection-cooked meat [184], though in raw meat, ultrasound treatment had no effect on total and soluble collagen contents [229, 230] or insoluble collagen [231].

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44 Ohmic Heating and Food Preservation

Marybeth Lima and Mohammad Shafiur Rahman

CONTENTS

44.1 OVERVIEW

Ohmic heating (OH), also known as Joule heating, electric resistance heating, direct electric resistance heating, electroheating, and electroconductive heating, is a process in which alternating electric current is passed through food material; heat is internally generated within the material due to its resistance to the applied electrical current. In conventional heating, heat transfer occurs from a heated surface to the product interior by means of convection and conduction and is time-consuming, especially with longer conduction or convection paths that may exist in the heating process. Electro-resistive or ohmic heating is volumetric in nature and thus has the potential to reduce over-processing by virtue of its inside–outside heat transfer pattern.

Ohmic heating is not a new technology; it was used as a commercial process in the early 20th century for the pasteurization of milk [1]. However, the "Electropure Process" was discontinued between the late 1930s and 1960s, ostensibly because of the prohibitive cost of electricity and a lack of suitable electrode materials. Interest in ohmic heating was rekindled in the 1980s when investigators were searching for viable methods to sterilize effectively liquid–large particle mixtures, a scenario for which aseptic processing alone was unsatisfactory. The purpose of this chapter is to present general information about ohmic heating, especially with regard to food preservation and processing.

44.2 GENERAL INFORMATION ON OHMIC HEATING

44.2.1 Advantages

Ohmic heating exhibits several advantages with respect to conventional food-processing technologies (Table 44.1).

44.2.2 Commercial Applications

OH can be applied to a wide variety of foods, including liquids, solids, and fluid–solid mixtures, and it is used commercially to produce liquid egg products in the United States. It is also used in the United Kingdom and Japan for the processing

TABLE 44.1
Advantages of the Ohmic Heating Process

1. Particulate foods up to 1 in^3 are suitable for ohmic heating; the flow of a liquid–particle mixture approaches plug flow when the solids content is considerable (20–70%).
2. Liquid–particle mixtures can heat uniformly (under some circumstances, for example, if liquids and particles possess similar electrical conductivities, or if properties such as solids concentration, viscosity, conductivity, specific heat, and flow rate are manipulated appropriately).
3. Temperatures sufficient for UHT processing can be rapidly achieved.
4. Absence of hot surfaces for heat transfer, resulting in a low risk of product damage from burning or over-processing.
5. Higher temperatures in particulates than liquid can be achieved, which is impossible for conventional heating.
6. Reducing risks of fouling on heat transfer surface.
7. Ease of process control with instant switch-on and shut-down.
8. Reducing maintenance cost (no moving parts).
9. High energy conversion efficiency.
10. Relatively low capital cost.

of whole fruits such as strawberries. Additionally, OH has been successfully applied to a wide variety of foods in the laboratory, including fruits and vegetables, juices, sauces, stews, meats, seafood, pasta, and soups. In 1997, there were 19 plants operating worldwide using ohmic heating technology [2].

The widespread commercial adoption of ohmic heating in the United States depends on regulatory approval by the FDA, a scenario that requires a full understanding of the ohmic heating process. This includes heat transfer (temperature distributions), mass transfer (concentration distributions, which are influenced by electricity), momentum transfer (i.e. fluid flow), and kinetic phenomena (i.e. thermal and possibly electro-thermal death kinetics, and nutrient degradation). Currently, there are no ohmic heating units available for household use.

Larkin and Spinak [3] examined safety considerations for ohmically heated, aseptically processing, multiphase low-acid food products and discussed the need for providing information on equipment design, product specification, process design, and process validation for regulators. Full knowledge of these areas is critical to ensure that the food product receives adequate thermal treatment. Significant research strides toward widespread commercial use are in progress.

44.2.3 Heat Generation

OH devices consist of electrodes, a power source, and a means of confining the food sample (for example, a tube or vessel). Appropriate instrumentation, safety features, and connections to other process unit operations (e.g. pumps, heat exchangers, holding tubes) may also be important. Ohmic heaters can be static (batch) or continuous. Figure 44.1 is a schematic diagram of a static ohmic heating process.

The key to the OH process is the rate of heat generation, the electrical conductivity of food material, and the way the food flows through the heater. The heat generation by electrical energy due to electrical resistance can be written as

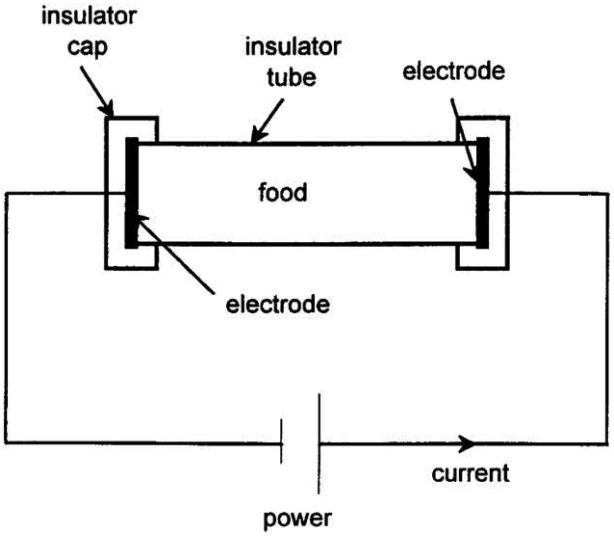

FIGURE 44.1 Schematic diagram of an ohmic heating process.

$$Q = I^2R = \sigma V^2 \tag{44.1}$$

where Q is the heat generation (W), I is the current (A), V is the voltage gradient (volt), σ is the electrical conductivity (S/m), and R is the electrical resistance (ohm). The electrical conductivity and resistivity from Ohm's law can be written as

$$R = \frac{V}{I} \qquad \text{and} \qquad \sigma = \left(\frac{1}{R}\right)\left(\frac{L}{A}\right) \tag{44.2}$$

where L is the length (m), and A is the cross-sectional area (m^2). The rate of heat generation depends on the local electric field strength. When a two-phase mixture is heated electrically, the two phases generate heat at the same rate if the liquid and solid have the same electrical resistance. When the fluid is of a higher electrical conductivity than the particles, non-uniform heating can occur in the fluid surrounding the particles if heating takes place under static conditions. The ratio of heat generation in solid to liquid is

$$\frac{Q_s}{Q_L} = \frac{\rho\sigma_s\sigma_L}{\left(\sigma_s + 2\sigma_L\right)^2} \tag{44.3}$$

where ρ is the density (kg/m^3), σ_s and σ_L are the electrical conductivity of solid and liquid, respectively, and Q_s and Q_L are the heat generation in solid and liquid phases, respectively.

Important design considerations include electrode configuration (current flows across the product flow path or parallel to the product flow path), the distance between electrodes, electrolysis (metal dissolution of electrodes, particularly at low frequencies), heater geometry, frequency of alternating current, power requirements, current density, applied voltage, and product velocity and velocity profile. Additional factors regarding the food system used in an ohmic heater include the type of product and its properties, especially electrical

conductivity and heating rate; others include percent solids, acidity, product viscosity, specific heat, and density, and solid particle size, shape, and orientation to the electric field. Substantial literature has been devoted to these topics [4–12].

Electrode design is a critical factor for the designing of OH processes. At least two or more electrodes are used to impart current in the fluid, and their locations or positions depend on the types of design. Two common designs are either static systems in a vessel, or continuous flow [13]. The open geometry types are easy to clean and reduce the effect of fouling with low damage to the products [14]. OH is usually arranged in one of four different configurations [12]. These are parallel plate configuration (transverse configuration), parallel rod design, collinear design, and staggered rod arrangement. Different conductive electrode materials, such as titanium, stainless steel, platinized-titanium, aluminum, and graphite, have been used for electrode. The selection depended on the price, corrosion resistance, and quality of the products. In addition, stainless-steel electrodes are preferred for quality products, and the frequency of the power supply must be increased to prevent corrosion and metal dissolution [15]. Coated electrodes can minimize or eliminate electrolytic reactions.

Using a 60 Hz sinusoidal alternating current, all the electrode materials exhibited intense electrode corrosion at pH 3.5 compared to other pH values, and titanium electrodes showed a relatively high corrosion resistance. The corrosion rate of other metals at pH 3.5 was about 55 times higher than that of titanium [16]. In the case of pomegranate juice, applying voltage gradients in the range of 30–55 V/cm showed that as the voltage gradient increased, the time of heating and pH decreased [17]. When the frequency of heating sample increased from 50.0 to 10×10^4 Hz, the time required for the heating sample to reach 80°C increased approximately six-fold [18]. Conventional OH under typical low-frequency alternative current of 50 to 60 Hz could cause oxygen and hydrogen evolution due to the electrolysis of water [19].

Temperature measurement remains an area of concern because many measurement methods influence the electric field during OH. A triple-point probe is the most satisfactory thermocouple for OH applications [20]. Some success has been seen with thermocouples that are coated with material, such as Teflon, however non-invasive temperature measurements that do not interfere with the electric field remain a challenge, particularly with regard to temperature measurement inside particles. Flash magnetic resonance imaging (MRI) could be used to determine rapid food particle temperature mapping during ohmic heating [21].

Heat is generated internally during OH, and this heating is not necessarily uniform. Thus, prediction equations to estimate heat generation are an important issue in OH to address questions like (i) where is the cold spot in the medium? (ii) What is the lethal treatment delivered to the cold spot? (iii) How is the lethal treatment ensured? Keys to OH include the rate of heat generation, the electrical conductivity of the food material, and the way in which food flows through the ohmic heater [3].

44.2.4 Cost

The economic analysis could play an important role in understanding the overall cost and viability of the commercial applications [12]. Investigators compared the cost of installation and operation of ohmic food-processing systems to that of conventional retorting, freezing, and heating in a conventional tubular heat exchanger [22]. The components included in the cost analyses were labor, energy, packaging, equipment maintenance and repairs, plant supplies, and interest and depreciation on the processing and filling equipment. Ohmic operational costs were found to be comparable to those for freezing and retort processing of low-acid food products. Though ohmic heating was found to be more costly than conventional methods for processing high-acid foods, still OH is viable considering other advantages.

44.3 PARAMETERS OF IMPORTANCE IN OHMIC HEATING

44.3.1 Electrical Conductivity

The most important parameter of interest in ohmic heating is the electrical conductivity of the food and/or food mixture. Substantial research was conducted on this property in the early 1990s because of the importance of electrical conductivity with regard to heat transfer rate and temperature distribution. The findings of numerous electrical conductivity studies are as follows: the electrical conductivity is a function of food components; ionic components (salt), acid, and moisture mobility increase electrical conductivity, while fats, lipids, and alcohol decrease it. Electrical conductivity is linearly correlated with temperature when the electrical field is sufficiently high (at least 60 V/cm). The increase in temperature increases the electrical conductivity due to the increase in ionic mobility [23]. Non-linear shapes (sigmoidal curves) are observed with lower electrical field strength [4, 18]: (i) electrical conductivity increases as temperature and applied voltage increases, and decreases as solids content increases, (ii) lowering the frequency of alternating current during ohmic heating increases the electrical conductivity, (iii) the waveform can influence the electrical conductivity; though alternating current is usually delivered in sine waves, and sawtooth waves increased the electrical conductivity in some cases, while square waves decreased it [18], and (iv) electrical conductivity increases by heating cycle; preheated samples show increased electrical conductivity as opposed to raw samples when both are subsequently subjected to ohmic heating [24].

The electrical conductivity of solids and liquids during ohmic heating of multiphase mixtures is also critically important. In an ideal situation, liquid and solid phases possess essentially equal electrical conductivities and thus (generally) heat at the same rate. When there are differences in the electrical conductivity between a fluid and solid particles, the particles heat faster than the fluid when their conductivities are lower than the fluid. Solid particulates heat more slowly than a fluid when the electrical conductivity of the solid is higher

than that of the fluid. Fluid motion (convective heat transfer) is also an important consideration when there are electrical conductivity differences between fluids and particles.

The current density (i.e. ratio between the current and electrode surface area) is important in calculating the critical current density, which is used in the design of the electrode dimension [13]. The voltage gradient increases the heat generation per unit time because of the resistance to current passing through the sample at any power applied, and it is related to the sample composition and its electric conductivity [20, 25–27].

Other product properties that may affect temperature distribution include the density and specific heat of the food product. When solid particles and a fluid medium have similar electrical conductivities, the component with the lower heat capacity tends to heat faster. High densities and specific heats are conducive to slower heating. Fluid viscosity also influences ohmic heating; higher viscosity fluids tend to result in faster ohmic heating than lower viscosity fluids [28].

44.4 MODELING OF OHMIC HEATING PROCESSES

44.4.1 Process Considerations

Considerable effort has been expended to model the heat transfer mechanisms and microbial death kinetics involved during ohmic heating. Models are of interest in the analysis and design of ohmic heating processes to provide information about the temperature distribution throughout the process, especially *cold spot*, and to provide accurate predictions of the minimum lethal processing time. Complexities in modeling heat transfer processes during ohmic heating arise when the liquid and particle possess different electrical conductivities, and because electrical conductivity is a (sometimes non-linear) function of temperature and frequency of alternating current. The thermal history and location of the *cold spots* or *cold zones* during OH require special consideration as the current knowledge of conventional heating cannot be extrapolated [23, 29].

Numerous models have been developed based on numerical solutions using transport equations with appropriate boundary conditions, assumptions, and dimensional groupings. These models contribute significantly to the understanding of heat transfer in OH. The voltage field (Laplace) equation for a single solid particle in a static heater has been solved [30]. Numerical solutions and experimental simulations of more complex ohmic heating situations have been developed [31–33]. Thermal-hydraulic aspects of ohmic heating were studied, and it was found that the temperature distribution can vary significantly even when ohmic heating rates are uniform [34]. Davies et al. [35] quantified the effects of a non-uniform electric field on temperature distributions during ohmic heating, while others [8, 36, 37] used magnetic resonance imaging to rapidly map and model the temperature of solid–liquid particle mixtures during ohmic heating.

A particle does not heat uniformly during ohmic heating because of the non-uniform nature of the electric field within the system [3]. Zones of high and low heat generation in a fluid around a particle of low electric conductivity are shown in Figure 44.2. The current is high on the sides of the particle and low in the front of rear regions, since current attempts to bypass the solid. This non-uniformity of heating can be reduced by increasing fluid mixing, reducing the viscosity of fluids, improving the rotation of particles, and the presence of other particles in the medium [38]. Kamonpatana et al. [39] developed mathematical models and microbiological verification of OH of a multicomponent mixture of particles (i.e. five types of particles) in a continuous-flow ohmic heating system with the electric field aligned parallel to flow. A chicken piece was found to be the slowest-heating particle in the worst-case simulation. The developed model and microbiological verification strategy could help to ensure sterility in a continuous-flow ohmic sterilization process for a multicomponent mixture.

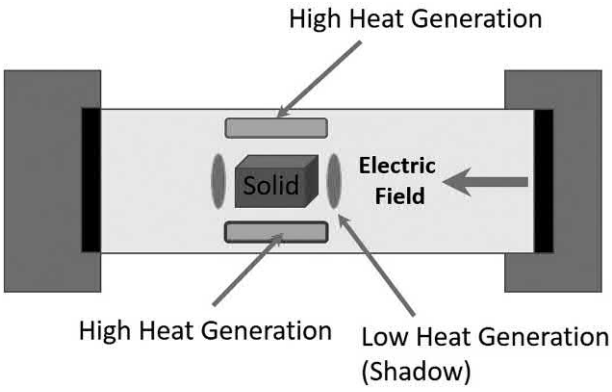

FIGURE 44.2 Zones of high and low heat generation in the fluid around a particle of low electrical conductivity. (Redrawn from Sastry [38].)

A dual cylindrical microwave and ohmic combination heater was developed for the minimization of thermal lags in the processing of particulate foods [40]. Temperature profiles of particle liquid mixtures containing sodium chloride solutions (5–20 g/L); chicken and potato particulates at different mass fractions (10–15 g/100 g) and sizes (0.5 and 1 cm cubes) were compared using a combination of heating modes. In the case of OH, particle size and salt concentration affected temperature variations between solution and particulates. However, the solution temperature in microwave heating lagged the particle temperature up to salt concentration 12.5 g/L regardless of the particle size and mass fraction, whereas the opposite trend observed at 20 g/L (i.e. very high concentration). The maximum temperature lags in the particulates were 7.1, 11.9, and 3.1°C, respectively, for microwave, ohmic, and combined heating, and a developed regression model could be used for the prediction of the temperature gradient as a function of salt concentration, particulate size, and concentration.

44.4.2 Microbial Death Kinetics

In terms of microbial death kinetics, considerable attention has been paid to the following question: does electricity result

in microbial death, or is microbial death caused solely by heat treatment? The challenge in modeling microbial death kinetics is the precise matching of time–temperature histories between ohmic processes and conventional processes. The FDA has published a comprehensive review of microbial death kinetics data regarding OH [41].

Initial studies in this area showed mixed results, though the experimental details were judged insufficient to draw meaningful conclusions [1]. Researchers compared the death kinetics of yeast cells under time–temperature histories as identical as possible and found no significant difference between conventional and ohmic heating except at low temperatures [42] (Tables 44.2 and 44.3). The decimal reduction times of *Bacillus subtilis* spores were significantly reduced when using ohmic heating at identical temperatures [43]. These investigators also used a two-step treatment process involving ohmic heating, followed by holding, followed by heat treatment, which accelerated microbial death kinetics; they hypothesized that electroporation may positively influence microbial death kinetics. The inactivation of yeast cells in phosphate buffer by low-amperage direct current electrical treatment and conventional heating at isothermal temperatures was examined [44]. These researchers concluded that a synergistic effect of temperature and electrolysis was observed when the temperature became lethal for yeast.

Tian et al. [45] performed OH (i.e. high-voltage-short-time (HVST) and low-voltage-long-time (LVLT)) and conventional water bath (WB) heating to identify whether a non-thermal death effect existed in OH. The heating on inactivation and proteome changes of *Escherichia coli* O157:H7 cells at the same endpoint temperature of 72°C (i.e. heating time varied) were measured. The inactivation of HVST was comparable to WB, and the largest inactivation was observed at LVLT, and there was lower intracellular protein content detected in LVLT and HVST samples than those of WB. Quantitative proteomic profiles identified 2626 proteins, and more protein changes in HVST and LVLT samples were mainly attributed to the leakage of intracellular proteins due to the damage of the cell membrane. In addition, bioinformatics analysis indicated that the differential proteins were mainly involved in transcription, translation, cell wall and membrane biogenesis, and amino acid, carbohydrate, and lipid metabolism. It was observed that 34 differential protein pathways were enriched, and five significant pathways were mostly related to the ribosome, terpenoid backbone biosynthesis, glycerophospholipid metabolism, ABC transporters, and folate biosynthesis. Therefore, additional non-thermal effects on the microbial inactivation existed.

Kim et al. [46] applied low-frequency (i.e. 0.06–1.0 kHz) pulsed OH (waveforms: sine, square, triangle, and pulse; titanium electrode) for the inactivation of foodborne pathogens (*E. coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes*) and bacteriophage MS-2 in buffered peptone water and tomato juice. In the case of pathogenic bacteria, they observed lower sub-lethal injury at 0.06–0.5 kHz than at 1 kHz, however MS-2 phage was inactivated more effectively at low frequencies and was more sensitive to acidic conditions than pathogenic bacteria. The pulsed OH waveform prevented electrode corrosion sufficiently at low frequencies (0.06–0.2 kHz). This process showed no electrode corrosion (pulsed wave) or quality degradation of tomato juice regardless of frequency. The increased electroporation effect at low frequencies was suggested as a reason for the reduced resuscitation level. Kim et al. [47] developed continuous OH (electric field strength 26.7 V/cm; frequency 25 kHz) at 85–100°C for 30–90 s on commercially processed apple juice using five sequential electric fields resulting in the rapid inactivation of *Alicyclobacillus acidoterrestris* spores. They suggested that the OH system is superior to conventional heating for rapid sterilization (30 s) of apple juice to assure microbiological quality without damage to the °Brix and color.

The heating rate and reduction rate of pathogens increased corresponding to decreased flow rate (i.e. higher numbers of pathogens survived at a higher flow rate). Increasing treatment voltage can be an effective way to inactivate pathogens, but the heating rate overly accelerates and adversely affects food quality (for example, color and lycopene retention in tomato juice). Kim et al. [48] proposed to increase the initial temperature by preheating, and this can help inactivate pathogens in the early treatment stage without affecting the heating rate. The flow rate, voltage, and initial temperature are important factors for determining pathogen inactivation performance of continuous-type OH.

Further research regarding microbial death kinetics, survivor counts, subsequent treatment, and the influence of electricity on cell death kinetics are necessary to address regulatory issues. It is commonly assumed that microbial death is

TABLE 44.2
***D* and *z* Values of Yeast Cells while Maintaining Identical Temperature Histories in Conventional and Ohmic Heating**

	D (s) Value at Different Temperatures				
Treatment	**48.8°C**	**52.3°C**	**55.8°C**	**58.8°C**	***z* value (°C)**
Conventional	294.6	149.7	47.2	16.9	7.2
Ohmic	274.0	113.0	43.1	17.8	7.7

Source: Palaniappan et al. [42].

TABLE 44.3
***D* and *z* Values of *E. coli* in Conventional and Electrical Pretreatment**

	D (s) Value at Different Temperatures				
Treatment	**60.0°C**	**64.5°C**	**68.5°C**	**71.0°C**	***z* value (°C)**
Conventional	164.8	48.2	23.0	11.8	9.9
Ohmic	180.4	51.2	21.2	11.8	9.4

Source: Palaniappan et al. [42].

only a function of temperature (heat) results, and this is now an appropriately conservative design assumption.

44.4.3 Vitamin Degradation Kinetics

Limited information exists regarding product degradation kinetics during ohmic heating. Researchers measured vitamin C degradation in orange juice during ohmic and conventional heating under nearly identical time–temperature histories and concluded that electricity did not influence vitamin C degradation kinetics [49]. This study was conducted at one electrical field strength (E = 23.9 V/cm). Others found that the ascorbic acid degradation rate in buffer solution during OH was a function of power, temperature, sodium chloride concentration, and products of electrolysis [11]. The *D*-values of ascorbic acid degradation in acerola pulp during OH were 30.2 and 38.0 h (temperature: 85°C), respectively at electric fields 10 and 100 Hz, and it was 38.6 h in the case of conventional heating [50]. The use of low electric field frequency (10 Hz) led to greater ascorbic acid degradation and higher color changes probably due to the occurrence of electrochemical reactions. Above 100 Hz up to 10^5 Hz (i.e. high electric field frequency) as tested, ohmic and conventional heating processes showed similar degradation rates of ascorbic acid and similar color changes. It was suggested that the ascorbic acid molecule predisposition for hydrogen donation in redox reactions was not affected by the rapidly varying electric field [50].

44.4.4 Mode of Actions

Early research on OH was conducted on heat transfer and the sterilization of liquid–particle mixtures. In executing such studies, investigators observed unanticipated phenomena. For example, OH beetroot resulted in enhanced diffusion of betanin from the beetroot tissue when compared to beetroot tissue heated conventionally [51]. These investigators hypothesized that the enhanced mass transfer could be caused by electro-osmosis.

Investigators expanded on the aforementioned work and found that the diffusion of beet dye from beetroot into a carrier solution was enhanced as much as 40% during heating from 20 to 80°C, and that the concentration of diffused dye was proportional to particle surface area and a linear function of electric field strength [24]. In the case of OH Japanese white radish, the heating rate was influenced by frequency; as the frequency of alternating current decreased, the heating rate increased [52]. These investigators used H-NMR analysis and hypothesized that at low frequency (50 Hz), rapid heating is caused by electroporation of the radish tissue membrane, which resulted in a decrease of electrical impedance. Subsequent studies have concluded that electroporation is the most likely mechanism for enhanced mass transfer effects during ohmic heating [10, 53].

Electroporation is defined as the formation of holes in a cell membrane resulting from the local pressure of ions, which cannot initially permeate the cell membrane but are forced against it by the electric field [54]. The relatively low alternating frequencies employed during OH enable this charge build-up to occur on the cell wall, resulting in the formation of pores. This also suggests that the lower the frequency of OH, the more pronounced the mass transfer effect; this concept has been demonstrated in the literature [55–57]. It was found that direct current resulted in less mass transfer enhancement than low AC frequency OH (at 15 V/cm, 250 Hz < DC < 50 Hz). In addition, it was postulated that a monopolar electric charge (DC) is not as effective as a bipolar electric charge at creating stress on the cell membrane, thus yielding less of an effect than low-frequency alternating current [55]. A moderate electric field enhanced the membrane permeability of potato cells and the electric field strength, temperature, and processing time. The type of plant tissue affected the degree of tissue damage and the extraction yield. It is believed that the electric breakdown and electroporation are the dominant mechanisms for the controlled low temperature, and higher temperature reduces non-thermal impact [58]. Electroporation also occurs in microbial cells [59].

Uniform and quick pH reduction (i.e. acidification) is important to achieve in foods, either treated by heat or low-pH foods. Conventionally, food-grade acids are used to modify the original pH, but it is a slow process. For instance, finished pH is achieved after 10 days [60]. However, vegetable acid-soaking treatments are carried out at refrigerated conditions for more than 24 h to control microbial growth [61]. It is also relatively slow. Blanching in acid solutions is another commonly employed method; however, it results in the leaching out of solutes. Considering these constraints, a combination of vacuum, high pressure, and OH could be used to reduce pH instantaneously and uniformly [62].

Electrically heating foods influences their mass transfer properties. This phenomenon has important implications for food-processing operations that involve mass transfer. In 2001, the FDA reported that "A large number of potential future applications exist for ohmic heating, including its use in blanching, evaporation, dehydration, fermentation, and extraction" [41].

44.5 APPLICATIONS OF OHMIC HEATING

44.5.1 Maintained Texture and Flavor

Sensory evaluation is critically important to any viable food process. Numerous publications have cited the superior product quality that can be obtained through decreased processing times, though few published studies specifically quantify sensory and texture issues. Six stew formulations sterilized using OH before and after three years of storage were analyzed; the color, appearance, flavor, texture, and overall food quality ratings were excellent, "indicating that OH technology has the potential to provide shelf-stable foods closely equivalent to those prepared from scratch." The quality and mechanical properties of hamburgers cooked with a combination of conventional and OH were not different from hamburgers cooked with conventional heating [36, 63].

In the case of OH (45, 60, 80 V at 60 Hz and 10, 100, 1000 Hz with 25 V, 65°C/30 min) of whey acerola-flavored drink, a similar fatty acids profile and volatile compounds were observed as compared to conventional heating. However, their intensity varied as a function of the parameters used (voltage and frequency) and resulted in more viscous beverages [64].

44.5.2 Gelatinization

Starch gelatinization is an important parameter in food processing and can be either advantageous or disadvantageous depending on the desired product formulation. The electrical conductivity of a food product is influenced significantly by starch gelatinization [65]. These investigators found that electrical conductivity decreased with the degree of gelatinization, and suggest that OH can be used in the development of a sensor to detect starch gelatinization. OH was used to maximize the gel functionality of a seafood product [66]. The OH process was superior to the conventional heating process because of rapid heating that deactivated enzymes, which in turn enabled strong gel formation.

44.5.3 Blanching

Because blanching requires large volumes of water during processing and often requires dicing vegetables, studies to increase the efficiency of blanching using OH are important. Wigerstrom [67] found that electric fields enhanced moisture loss during the blanching of potato slices. Mizrahi [68] determined that OH was an effective method for blanching because the rapid, uniform heating exhibited by OH eliminated the need for dicing vegetables. The quick process time and reduction in surface area (no dicing) reduced solute losses by an order of magnitude during blanching. Sensoy and Sastry [69] found that using ohmic heating during the blanching of mushrooms resulted in the shrinking of mushrooms at a lower temperature and with less water use as compared to conventional blanching. Lakkakula et al. [56] showed significant lipase deactivation in rice bran during ohmic heating, with and without a corresponding temperature increase. Taken collectively, these studies show that ohmic heating can increase process efficiency in blanching. Considering OH and conventional blanching (CB) of pumpkin, peroxidase inactivation kinetics and color changes were measured [70]. It was verified that OH accelerated the enzymatic inactivation process; for a reduction higher than 90% (i.e. peroxidase) it took 2 min for OH whereas CB took 4 min with no significant difference in color changes between the processes.

Considering apple juice, OH of polyphenol oxidase inactivation was optimized considering temperature (60–80°C) and voltage gradient (30–40 V/cm) [71]. An improvement in apple juice quality was achieved by using OH at 80°C and 40 V/cm as compared to CH. The total extracted phenolic content was increased by 5.4% with OH and 2.5% with CH as compared to fresh apple juice. The color was improved and loss of ascorbic acid and carotenoids decreased in the case of OH.

However, there was an absence of non-thermal effects of electricity (frequency 10–10^5 Hz; waveforms, sine, square, triangle, and pulsed; voltage gradients, 3.9 and 20.5 V/cm; temperature 75°C for 25 min) on the enzymatic inactivation kinetics of peroxidase and phenolic compounds degradation in the case of sugarcane juice. Selected waveforms promoted higher degradation of these compounds, and color changes were observed when 10 Hz/25 V and 60 Hz/130 V were applied, with higher electric field strength [72].

44.5.4 Peeling

Peeling is one of the most important preparatory steps in the processing of fruits and vegetables for canning and other methods. In the case of OH-assisted lye peeling, it was observed that the concentration of lye (2% sodium hydroxide at 532 V/m and 3% sodium hydroxide at 426 and 479 V/m) can be significantly lowered in the presence of OH to achieve high peeled yields and quality, which is considered the complete removal of peels [73]. In addition, the effects of increasing field strength yielded no significant additional benefits.

44.5.5 Evaporation

Wang and Chu [74] studied the effect of OH on the vacuum evaporation of orange juice and found that the evaporation rate could be increased as much as three times using ohmic heating, resulting in enhanced product quality. The authors concluded that OH could have potential as a fast evaporation method and recommend further development in this area. OH was applied in a seawater desalination thermal process (i.e. water is evaporated from seawater by heating and then condensed to produce pure water) with the advantage that there was no fouling on the conventional heat transfer surface although some issues of color existed [11, 75]. OH was also applied in hydro-distillation, for example, extraction of essential oils and alcohol concentration [76, 77]. The emerging OH distillation method was shown to be a potential alternative to hydro-distillation for the recovery of target components from miscible and immiscible mixtures such as ethanol–water and aromatic herbs–water, respectively. It offers several advantages such as better process control, reduced distillation cost, and shorter process time without adversely affecting the yield and distillate quality [78].

44.5.6 Dehydration

Ohmic heating was used to enhance the drying rate of vegetable tissue. OH of sweet potato prior to dehydration accelerated the hot-air drying rate significantly as compared to raw, conventionally treated, and microwaved samples [79]. Ohmic pretreatment accelerated the vacuum drying rate of sweet potato as much as 24%; and minimal ohmic treatment (electrical field strength of 50 V/cm and an endpoint temperature of 40°C) resulted in the maximal or near-maximal acceleration of drying rate [80].

Lima and Sastry [57] found that the lower the frequency of alternating current used in OH, the faster the hot-air drying rate. Maximum drying benefits were seen when drying

to initial or intermediate moisture contents. These investigators suggested that because OH enhances drying rates and enhances extraction yields, the process could be ideal for the recovery of high-value, heat-labile components from biological materials using unit operations such as supercritical fluid extraction. Moraveji et al. [81] simulated the transport phenomena of the potato drying process in a static system considering electrical field intensity, electrical conductivity, solid heating, liquid–solid conductivity, and predicted the parameters, such as drying rate, moisture content, and temperature changes, on the drying process of potato. It was observed that OH reduced the drying time and accelerated the diffusion in the entire product as compared to other conventional methods. In addition, heat and mass transfers simultaneously occurred nearly at the same rate in both phases (i.e. solid and liquid).

44.5.7 Fermentation

Cho et al. [82] found that mild electrical treatment significantly decreased the lag time of *Lactobacillus acidophilus*, possibly due to electroporation, which could enhance the transport of substrates across the cell membrane. The electricity applied later in the microbial growth cycle proved detrimental, possibly because of the enhanced transport of inhibitory substances across the cell membrane.

44.5.8 Enhanced Growth of Beneficial Bactria

Considering yogurt, OH under sub-lethal conditions presents promising results regarding the enhancement of growth rate (i.e. enhanced metabolism), whey protein structure as a potential carrier of probiotic entities and bacteriocin activity, leading to considerable improvements in the fermentation process [83]. However, Pereira et al. [83] pointed that fundamental knowledge is still required to understand interactions between electric fields and whey protein structures, and the interaction between alternating electric fields and the microorganism is far from being understood.

44.5.9 Extraction

Ohmic heating has been used to enhance the extraction of components from foods. For example, an electric field was used to extract sugar from sugar beets [84] and soymilk from soybeans [85]. OH of apple tissue prior to mechanical juice extraction significantly increased apple juice yields with respect to un-treated apple tissue, and the lower the frequency of alternating current, the higher the extraction yield [57, 86]. Similarly, a significant increase in the extraction of rice bran oil from rice bran (with moisture addition) was observed, especially at low (1 Hz) frequency [56].

Several studies have examined the diffusion of beet dye from beetroot. The diffusion enhancement of beet dye due to ohmic heating was especially pronounced at lower temperatures (42°C vs. 58°C and 72°C) and could be related to the difference in electrical conductivity of beet tissue between conventional and ohmic cases at the same temperature [87]. Kulshrestha and Sastry [53] showed that significant leaching of beet dye occurs with temperature increases of 1–2°C in OH. OH was applied to extract inulin from the wet-milled and dry-milled powders of Jerusalem artichoke tuber, and it was observed that OH provided a higher inulin extraction yield than the conventional heating method. Furthermore, dry milling produced a higher extraction yield and inulin purity than wet milling [88]. The juice yield and bioactive compounds of apple and carrot mashes treated by PEF or OH at different preheating temperatures (40, 60, or 80°C) were increased. A release of total polyphenols from apples into the juice increased in all pre-treated samples compared to the control. This was due to the thermal and electric field-based cell disintegration [89].

Moderate electric field extraction at low and high temperatures offers several advantages, such as enhancing product yield and quality with saving time and energy. Optimizing process parameters, such as treatment time and temperature, electric field intensity, and frequency, can boost the performance of such a system. In addition, the proper selection of raw material and preparation are needed [58]. Non-electroconductive materials such as glass, polyvinyl chloride, acrylic, Teflon, ceramic, and polypropylene were used for the extraction chamber [58].

44.5.10 Emulsion Stability

The stability of iron-loaded double emulsions was monitored under the influence of novel (microwave and ohmic) and conventional heat treatments [90]. Microwave heating led to destabilization and obvious phase separation. Considering iron release and color, conventional heating resulted in greater stability than OH. In addition, OH enhanced lipid oxidation (in yogurt as a model system) and increased iron release as compared to the conventional heating.

44.6 CONCLUSION

OH shows potential in food processing and preservation. The important factors that need to be considered for designing OH are electrode configuration, types of electrodes, electrical conductivity, applied voltage gradient, waveforms, frequency, fluid viscosity, solid–liquid ratio, and direction of fluid flow with respect to current flow. The mechanisms of action are electroporation and change in protein and starch configuration. OH is newly commercially used in many countries. Many applications, such as thermal treatment, bacterial inactivation and growth enhancement, peeling, blanching, drying, evaporation, distillation, and desalination, are now documented in the literature.

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45 UV Light in Food Preservation

Mohammad Shafiur Rahman

CONTENTS

45.1 ULTRAVIOLET RADIATION

Ultraviolet radiation (UV) has been known for long as the major factor in the bactericidal value of sunlight. It is mainly used in sterilizing air and thin films of liquid due to low penetration depth. When used at high dosage there is a marked tendency toward flavor and odor deterioration before satisfactory sterilization is achieved. The low level of radiation at carefully applied doses can often usually extend the shelf life of foods without serious change in quality [1]. The technique of UV radiation to kill off bacteria in water is well-known. UV is safe, environmentally friendly, and more cost-effective to install and operate than conventional chlorination. It does not affect the taste of the water as chlorine does. High-intensity UV-C lamps can increase the potential of destroying surface bacteria on food [2]. UV radiation has been used in dairy plants for many years. It is also used in the ice cream industry and in meat and vegetable processing plants [3]. More applications of UV irradiation are presented by Falguera et al. [4]. A cost comparison of UV with the other processes is given in Table 45.1.

45.2 UV MODE OF ACTION

A number of theories have been proposed with regard to the mode of action of UV light. These include indirect lethal action resulting from the production of hydrogen peroxide, and various chemical and physiochemical changes in the constituents of the cell. The production of hydrogen peroxide is not generally considered as the mechanism by which ultraviolet light induces its effect, although organic peroxides may be involved. It has been suggested that substances of the cell nucleus are involved in the destruction action by UV light. UV wavelengths of 200 to 290 nm penetrate cell membranes to disrupt DNA molecules, preventing cell replication [1]. In addition, the degradation of the bacterial cell walls can cause a germicidal effect [5, 6]. Photo-reactivation could also enhance the effect on microbial deactivation and other physicochemical changes.

45.3 UV IN FOOD PRESERVATION AND DETERIORATION

Current UV reactors for juice processing use either very long tubes with UV transparent walls or very thin films to ensure sufficient exposure to UV radiation. The reactor design with long tubes requires turbulent flow and over 38 liters of juice exposed to a large number of UV lamps. In contrast, the design with thin films operates in laminar flow but is restricted to low-viscosity juices with no pulp. Recently a reactor has been designed involving the pumping of fluid through the annular gap between two concentric cylinders. To provide sufficient exposure and to reduce the fluid boundary layer thickness next to the radiation source contained within the outer stationary cylinder, the smaller inner cylinder rotates at a low rpm. Rotation of the inner cylinder establishes a complex flow field called Taylor–Couette flow consisting of laminar vortices that both fill the annular gap of several millimeters and circumscribe the inner cylinder. UV is one of the non-thermal processes capable of providing the FDA requirement of a 5 log reduction in viable pathogens [7]. A numerical dispersed phase model has been developed to describe the particle-phase flow patterns and particle residence times in thin-film UV reactor Cider Sure 1500 [8]. This model was used to simulate the processing of apple cider. UV irradiation is applied commercially in food processing by bactericidal ultraviolet lamps: tenderizing or aging of meat, curing and wrapping of cheeses,

TABLE 45.1
Cost Comparison: Juice Processing Methods

Process	Cost (US cents/gal)
Carbon dioxide	5
High pressure	15
Pasteurization	5
Conventional UV	0.2

Source: Forney and Pierson [7].

prevention of surface mold growth on bakery products, and air purification in bottling and food processing establishments and over pickle vats [1].

It is generally agreed that the wavelength for maximum germicidal effect is 2600 Å. Low-pressure mercury-vapor lamps have a maximum output at 2537 Å, a value close to the peak wavelength for bactericidal effectiveness. The lethal action varies with the time of exposure and intensity of light. Other influential factors include temperature, hydrogen ion concentration, and the number of organisms per unit area exposed. The relative humidity affects the death rate of bacteria suspended in air, this being most noticeable at relative humidity values greater than 0.50, at which point an increase in relative humidity results in a decreased death rate [1]. Pulsed xenon UV systems can disinfect aerobic bacteria in the absence of manual disinfection [9].

Spores of bacteria are generally more resistant to UV light than vegetative bacteria; *Bacillus subtilis* is reported as five to ten times more resistant than *Escherichia coli*. Molds are more resistant than vegetative bacteria, while yeasts differ less from bacteria in this respect. It has been suggested that some mold species may be protected by fatty or waxy secretions on the cell surface that shield them from the rays. Pigments apparently also afford some protection; dark-pigmented spores are more resistant to UV irradiation than non-pigmented types [1]. Short exposures, even long enough to cover one or more life cycles of the organism, are more efficient than higher radiation intensities for brief periods. This presumably is due to the fact that during certain stages of the life cycle the susceptibility to ultraviolet radiation is increased.

45.3.1 Juice

The lethal action of ultraviolet light on microorganisms has been well-documented. The practical application of this has been controversial because of the type and intensity of radiation, methods of estimating lethality, and other factors. A study of the germicidal powers of UV shows that 30 to 83% of the yeast, and 33 to 72% of the molds, were killed in apple cider through layers varying from 2 to 25 mm in thickness [1]. A greater part of the light was absorbed by coloring matter. Incident energy levels of 254 nm inhibited 90% of *Bacillus megatherium* at 11 kW/m^2 and 90% of *Sarcina lutea* at 198 kJ/m^2 [5]. There was a 90% reduction in the microbial count of apple juice. Coupled with effective refrigeration, this could be of commercial significance. Inactivation of *E. coli* O157:H7 in apple juice showed 2.81 log, 1.95 log, and 1.85 log reduction when exposed to UV-C (254 nm), Far-UV (222 nm), and Far-UV (282 nm), respectively, at the similar levels of fluence of 750 kJ/m^2 [10]. *E. coli* O157:H7 was shown to be acid-resistant, and up to 99.5% of cells survived in apple juice when incubated at 20°C for 24 h, while no reactivation potential was observed in dark incubation phases after exposure to UV light. However, the effect of deactivation by Far UV (222 nm) was higher as compared to UV-C and Far-UV (282 nm) when stored at 37°C.

Maple sap is susceptible to microbial infection, which, when it occurs, lowers the quality of the syrup. Schneider et al. [11] studied the effects on the reduction of living cells of bacteria (*Pseudomonas-25* and *Pseudomonas-11*) and a yeast (*Cryptococcus albidus*) strain suspended in maple sap when exposed to UV radiations of different intensities and for different lengths of time. The two bacterial strains were equally more sensitive than yeast. An increase in exposure time had the same effect regardless of the method of irradiation employed.

Ultraviolet C treatment is an emerging food-processing technology for health-conscious consumers. Freshly squeezed mango juice was exposed to UV-C light (for 15, 30, and 60 min at 25°C) and thermally pasteurized (at 90°C for 60 s) to compare the effect on microbial inactivation, physicochemical properties, antioxidant activities, and other quality parameters. In addition, a shelf life study of juice samples stored at 4°C was conducted for 5 weeks. In the case of mango juice, UV-C treatment and thermal pasteurization (i.e. 90°C for 60 s) showed no significant changes in physicochemical properties. An increase in extractability of carotenoids (6%), polyphenols (31%), and flavonoids (3%) and enhancement of antioxidant activity were observed in the juice exposed to UV-C for 15 and 30 min at 25°C as compared to freshly squeezed juice [12]. Thermal pasteurization and UV-C treatment exhibited a significant reduction in microbial load and prolonged the shelf life of juice. The shelf life of UV-C-treated juice stored at 4°C was extended for at least 4 weeks longer than freshly squeezed juice.

Fresh pitaya juice showed a reduction of 1.81 log cycles of *Zygosaccharomyces bailii* when treated (flow rate 30.3 mL/s and time 30 min) with UV-C light treatment (0.57 W/m^2), while mesophylls showed 1.76 log cycles [13]. The treatment did not affect pH and total soluble solids, while the total color change was slightly affected by the flow rate and treatment time. The phenolic content was not affected by the treatment, while betalain content and antioxidant activity were reduced as the flow rate increased. The color parameters and betalains content of treated juice decreased after 10 days of storage at 4°C, while phenolic compounds remained constant during the entire storage time and antioxidant activity was reduced after 5 days of storage. The mesophylls and yeasts plus *Z. bailii* quasi remained constant during 10 days of storage.

Freshly pressed apple juice or fresh cider contains many microorganisms, which cause deterioration within 2 days

at room temperature unless they are inhibited or destroyed. The microbial population of fresh cider was greatly reduced and storage life was prolonged without affecting the flavor by specially designed UV lamps [14]. Harrington and Hills [14] found that the percentage reduction of microbial activity was affected by the clarity of the cider, the length of UV exposure, and the presence of potassium sorbate. This is very suitable where the initial microbial count is high and there is not adequate refrigeration. Hoyer [15] reported values of UV dose for 90% reduction of *E. coli* ATCC 11229 and *E. coli* ATCC 23958 were 25 and 12.5 J/m^2, respectively, and a value of 15 J/m^2 for a 1 log reduction of *E. coli* O157:H7 in drinking water. UV doses for a 90% reduction in model juices with dose distribution in annular UV reactors were determined and varied from 4.5 to 6.5 kJ/m^2 in a vertical set up [16]. The UV dose distribution must be taken into consideration when calculating the microbial inactivation achieved by the reactor and designing a continuous UV reactor [16].

UV (254 nm, bench-top, batch-scale unit with incident UV intensity of 200 W/m^2) exposure resulted in a more than 90% reduction in PPO activity in mushroom after 5 min of exposure (480 kJ/m^2) [17]. A model system of typical fruit and vegetable juice (glucose, sucrose, and fructose at 10 g/100 mL level, pH 3.5–6.8, and suspended particles 844 NTU) was used to treat PPO. A depth of 1.4 mm with gentle agitation was used. PPO inactivation was accelerated by fructose due to the generation of reactive oxidative species by fructose, while sucrose and glucose showed no effect on the inactivation rate. The inactivation rate was higher at pH 3.5 than at pH 6.8, and suspended particles reduced the inactivation due to the scattering of UV light. Additionally, no reactivation was observed during storage.

In the case of coconut water, 3.1–7.8 log microbial reductions were observed for the inoculated *Lactobacillus rhamnosus*, *Salmonella typhimurium*, and *Saccharomyces cerevisiae* after 10 min of treatment of UV-C light (52–264 kJ/m^2) [18]. The residual effect during storage at 5 and 25°C showed the bacterial decay when stored at 5°C; while 25°C storage showed maximum growth of 1.12 log (12 h storage) and 0.8 log (24 h storage) cycles for *L. rhamnosus* and *S. cerevisiae*, respectively. The residual effect showed that no refrigeration may be required for the storage of coconut water.

Iwanami et al. [19] studied the effect of UV radiation in a lemon flavor composed of lemon oil, water (pH 6 phosphate buffer), and ethanol. Three new compounds of aldehyde, newly identified as photoreaction products of citral, limonene, terpinolene, and nonanal, decreased, while p-cymene increased after UV radiation. Other components, such as sesquiterpene hydrocarbons, citronellal, linalool, and terpineols, were slightly changed. These results suggested that UV radiation is critical for the UV-unstable component in lemon flavor, and the photolysis of citral could affect other components in lemon flavor [20].

45.3.2 Fruits and Vegetables

The effect of UV on bacteria and fungi, such as *Penicilli* and *Aspergilli*, was reported by Kleczkowski [20]. UV inhibits the fungal development in grape berries [21], kumquat, and orange fruits [22]. Moy et al. [23] combined UV and gamma radiation for the preservation of papaya. Combined methods can avoid high doses of gamma and UV radiations. UV irradiation exhibits a hormetic effect in disease resistance and control of diseases for postharvest crops [24].

Lu et al. [25] studied the efficacy of gamma rays (0.1 to 3 kGy), electron beams (0.1 to 5.0 kGy), or UV radiation (0.44 to 7.33 kJ/m^2) to preserve onions for up to four weeks at 20–25°C. UV-radiated onions exhibited the greatest percentage of marketable onions and reduction in postharvest rots. Sprouting was observed with control, UV-, and electron beam-irradiated onions but not with gamma-irradiated onions. No significant total sugar, pH, moisture, ascorbic acid, color, texture, or sensory quality changes were observed with the onions irradiated with UV. The optimum UV doses were in the range of 3.58 to 7.33 kJ/m^2 for onions. In addition, UV is much more economical and safer to use than gamma or electron beam irradiation.

Ranganna et al. [26] studied the efficacy of UV treatment in the control of both soft rot and dry rot diseases of potato tubers for short-term storage of three months and to understand the tuber quality changes. They tried four UV radiation dose levels (7.5, 10.0, 12.5, and 15.0 kJ/m^2), and three incubation levels each for fungi *Fusarium solani* (0, 1, and 2 days) and bacteria *Erwinia carotovora* var. *carotovora* (0, 6, and 12 h). The highest UV dose level was found to be more effective as compared to the other three radiation dosages to control disease caused by the above fungi and bacteria. The visual observations of potato quality showed that there was no significant change in the tuber quality such as firmness and color.

The effect of a hormetic dosage of UV radiation in delaying the senescence of tomato was investigated [27]. Mature green tomato fruits were irradiated (UV-C, 200–280 nm) corresponding to 0, 3.7, and 24.4 kJ/m^2 and were stored at 16°C, under high relative humidity for a period of 35 days. Attributes of senescence such as weight loss, color, texture, respiration rate, ethylene production, and putrescine were monitored periodically throughout the storage period. A dose of 3.7 kJ/m^2 was found to be beneficial (hormetic) in delaying ripening and senescence, while the higher dose impaired ripening and caused abnormal browning, manifested as sunscalding of the fruit's surface. The development of color and softening of tissue were significantly retarded during storage in response to the treatment with the hormetic dose. In addition to a delay in the climacteric response by at least 7 days, the respiration rate and ethylene production of the treated fruit were also reduced. The delay in senescence was attributed, in part, to the maintenance of a high level of putrescine (antisenescence agents exerting an opposite physiological effect to ethylene) [27].

UV-C irradiation could be used as an effective and rapid method to preserve the postharvest life of ripe mangoes without adversely affecting certain quality attributes [28]. This was observed after 10- and 20-min exposure prior to storage for 14 days at 5 or 20°C and a shelf-life period of 7 days at 20°C. Treated fruit maintained better visual appearance,

suppressed decay symptoms, maintained firmness, showed greater levels of putrescine and spermidine, and retained higher levels of sugars and lower levels of organic acids during storage at 5 or 20°C.

Mau et al. [29] found that UV irradiation increased vitamin D_2 content in edible mushrooms. After UV-C irradiation for 2 h, vitamin D_2 contents in common and high-temperature mushrooms increased from 2.20 and 4.01 μg/g of dry weight to 7.30 and 5.32 μg/g, respectively. After UV-B irradiation for 2 h, the vitamin D_2 content in common mushrooms reached 12.48 μg/g. UV-B irradiation resulted in higher vitamin D_2 conversion for common mushrooms. After UV-B irradiation for 2 h, vitamin D_2 contents in shiitake and straw mushrooms increased from 2.16 and 3.86 to 6.58 and 7.58 μg/g, respectively. The increased rates in shitake and straw mushrooms were not as high as in common mushrooms.

The enzymatic browning in fruit and vegetable tissues containing phenolic or polyphenolic compounds is common. A reduction of 58.7% in polyphenol oxidase (PPO) activity was achieved in the first 90 s when UV-Vis irradiation was used, and it was completely inactivated after 35 min of treatment [30]. The melanin pigment was synthesized and absorbed some radiation energy, thus leading to a slower inactivation of polyphenol oxidase.

UV irradiation (6 to 48 h, 13.0–103.7 J/m^2) showed potential for increasing the mechanical strength of casted soy protein films [31]. Besides increasing tensile strength, the appearance of immobile bands in electrophoretic patterns suggested further development of covalent cross-links in UV-treated films. Individual proteins may exhibit different degrees of response to UV radiation due to varying amino acid compositions and molecular structures. UV treatment would not be expected to render soy protein films unsuitable for use as edible films or as food wraps. UV treatment increased the tensile strength of gluten, zein, and albumin films suggesting the occurrence of UV radiation-induced cross-linking within film structures. For caseinate films, UV-curing did not affect tensile strength but substantially reduced total soluble matter. Small but significant decreases in the total soluble matter were also noticed for UV-treated zein and albumin films.

UV irradiation reduced the water vapor permeability of albumin films but did not affect the water vapor permeability of the other types of films. Gluten, albumin, and caseinate films had increased yellowness as a result of UV treatment. In contrast, UV treatment decreased the yellowness of zein films, possibly due to the destruction of zein pigments by UV radiation [32]. The gel strength of minced mackerel with transglutaminase alone at a concentration of 0.47 unit/g was three times greater than that of control [33]. When transglutaminase-supplemented minced mackerel was exposed to UV light for the optimal irradiation time of 20 min, the gel strength could be further increased by 25%. This suggested that UV irradiation accelerated the transglutaminase to catalyze the cross-linking of myosin heavy chains in mackerel actomyosin [33]. With increasing time of exposure to UV radiation, the molecular weight of proteins in water solutions decreased with the excluded volume increases for the two kinds of proteins (pepsin and albumin) as studied by Maciejewska et al. [34]. The denaturation effect of dimethyl sulfoxide on the solid samples of both starch components (amylase and amylopectin) was demonstrated by its spectral similarity [35]. In a study by Fiedorowicz et al. [36], a suspension of corn starch in water was irradiated by UV light with a wavelength greater than 250 nm at 25°C, under a stream of nitrogen or air, for time intervals ranging from 5 to 25 h. They found that molecular size distribution profiles confirmed the photo-degradation to be oxidative in the early stage (up to 5 h), and cross-linking reactions in the later stage (5–15 h) of irradiation under aeration.

The pulsed UV light pretreatment reduced the water content in mangoes, but did not affect the apparent water diffusivity of the subsequent drying process [37]. Vitamin C and carotenoids in dried mangoes increased 10 and 40% (36 and 108 kJ/m^2) as compared to the untreated dried mango, while vitamins B_1, B_3, and B_5 increased by 10 to 25% (36 and 72 kJ/m^2). Vitamin B_6 was highly affected by pulsed UV light, decreasing by 40 to 50% in the pretreated mangoes.

45.3.3 Meat

After killing, meat becomes tender upon storage as a result of enzymatic activity. This process is speeded up at relatively high temperatures, which favor the growth of surface microorganisms. By controlling such growth with ultraviolet light, the advantage of the high storage temperature can be better utilized and result in less loss of meat. In this particular case, irradiation alone is the less likely active factor. The lamps employed emit rays not only in the germicidal 2537 Å range but also in the 1850 Å range. These shorter waves convert atmospheric oxygen to ozone; irregular and shaded areas of an irradiated surface are sterilized by the ozone. UV is also used in storage vats and other tanks, over both conveyers and for final treatment of both caps and stoppers [1].

Putrefaction spoilage of fresh meat can occur in a few hours as a result of the action of spoilage bacteria. UV at a wavelength of 254 nm was effective in destroying surface bacteria on fresh meat by 2 log cycle (99% reduction) after a radiation dose of 1.50 kW/m^2 on smooth-surface beef meat. A further increase in dose level to 50.0 kW/m^2 reduced 3 log cycle. Since UV radiation does not penetrate most opaque materials, it was less effective on rough surface cuts of meat, such as round steak, because bacteria were partly shielded from the radiation. No deleterious effects on color (redness) or general appearance were observed, and UV irradiation of meat carcasses could increase the lag phase of bacteria until adequate cooling of the surface [38].

The physical appearance of a retail product in the display case is the most important factor determining consumer selection of beef products. Reagan et al. [39] mentioned that significant increases in shelf life may be obtained by exposure of beef muscle and fat surface to UV light (max wavelengths 3660 Å for 2 min). UV treatment decreases initial count and/or attenuation of the bacteria present on retail cuts, and

resulted in increased consumer acceptability, higher muscle color ratings, and increased shelf life of beef [39].

Kaess and Weidemann [40] found that continuous UV ($0.2–24 \times 10^{-2}$ W/m^2) irradiation of psychrophilic microorganisms growing on muscle slices at 0°C and 0.993 equilibrium relative humidity resulted in an extension of the lag phase of *Pseudomonas* and of the molds *Thamnidium* and *Penicillium*, but not the yeast *Candida scottii*. A minimum intensity of 2.0×10^{-2} W/m^2 at the meat surface is necessary to prolong storage life substantially. Lower equilibrium relative humidity did not substantially increase UV effects. The relative extension of storage life at 10°C was comparable to that obtained at 0°C. The simultaneous use of UV (0.2×10^{-2} W/m^2) and ozone (0.5 mg/m^3) produced synergistic effects with molds, but not with bacteria [40].

45.3.4 Minimally Processed Fruits and Vegetables

UV-C radiation can be considered a promising tool for maintaining the overall quality of fresh-cut fruits if applied in mild doses [41, 42]. All treatments with UV-C (1.2–24.0 kJ/m^2) imparted the same germicidal effect with a 1–2 log reduction in total viable counts in fresh-cut apple [42]. The loss of compartmentalization of surface apple cells, activating dehydration and oxidative phenomena, was observed with treatments above 1.2 kJ/m^2. Mild treatments resulted in more stability in terms of microbial growth and the development of browning and off-flavors as compared to the untreated. In addition, an edible protective film formed during treatment inhibited microbial growth and hindered dehydration during storage, while this was too thin to be perceived visually. UV-C light (fluency: 0.2–4.8 J/m^2 and 200 J/m^2) did not decrease the initial microbial load of fresh-cut pineapple sticks [43]. However, treated pineapple packaged in conventional PET/EVOH/PE trays and stored at 6°C showed slower (i.e. 2 log cycle lower) yeast and lactic acid bacteria growth up to 15 days. In addition, UV-C light did not promote changes in color and increased consumer preference.

The microbial counts of fresh-cut watermelon reduced just after exposure, and after 11 days at 5°C, mesophilic, psychrophilic, and enterobacteria populations were lower in the UV-C-treated watermelon [41]. Slight changes in color were observed. Considering sensory quality, low-dose (1.6–2.8 kJ/m^2) treated fruit can be stored for up to 11 days at 5°C, while the maximum shelf-life of moderate- to high-dose (4.8–7.2 kJ/m^2) treated fruit was 8 days at 5°C. The untreated and UV-C-treated fruit showed a similar decrease in lycopene after 11 days' storage at 5°C, and the decrease was low at low doses. The vitamin C level remained the same, while total polyphenols content considerably declined throughout the storage period and total antioxidant capacity markedly increased. Higher doses induced slightly higher carbon dioxide production, while no changes in ethylene production were observed, and final gas partial pressures remained the same within modified atmosphere packages.

The water consumption in fresh-cut salad washing can be reduced by UV-C light [44]. Water drained from lettuce was collected and then treated with UV-C light (up to 1.2 kJ/m^2) and reused for up to five washing cycles. A considerable amount of UV-C light can penetrate wash water if its thickness is lower than 1 cm. A dose of 0.4 kJ/m^2 allowed the inactivation of most of the native microflora and the achievement of more than 5 log reductions in inoculated *Salmonella enterica*, *Listeria monocytogenes*, and *E. coli*. In multiple washing cycles up to 5, the treatment resulted in more than 3 log reductions in native microflora in the wash water.

45.3.5 Fish and Seafood

The use of UV radiation is effective in inhibiting the action of spoilage bacteria on fish and seafood [2]. UV at 254 nm and doses of 3.0 kW/m^2 from a photochemical reactor or 48 J/m^2 from a high-intensity UV-C lamp (40 sec at 120–1.8 kW/m^2) reduced the surface microbial count on mackerel by 2 or 3 log cycles [3]. Huang and Toledo [3] mentioned that the shelf life of Spanish fresh mackerel was extended by 7 days over the untreated sample when the skin surface was treated with high-intensity UV and stored in ice at –1°C. When UV irradiated and packed in 0°C ice, surface microbial counts on vacuum-packaged mackerel lagged by 4 days compared to those on mackerel wrapped in 1 mil polyethylene [3].

The use of UV has some disadvantages, such as it does not penetrate most opaque materials and it is less effective on rough surfaces [2]. Huang and Toledo [3] found that rough-surface fish such as croaker and mullet had little bacterial count reduction on its surface with a UV-C-13 lamp with doses of 1.2 to 1.8 kW/m^2 up to 50 s treatment. They found that spray washing with water containing 10 ppm chlorine by itself or in combination with UV was necessary to reduce surface counts on rough-surface fish to the same extent as that on smooth-surface fish.

UV at 260 nm (6 W/m^2) could inactivate mold *Aspergillus niger*, *Penicillium citrinum*, and *Cladosporium cladosporioides* in dried fish products, such as dried filefish fillets without any concomitant changes in the color or sensory qualities [45]. The overall reduction of the spoilage molds ranged from 1 to 2 log cycle (6–18 W/m^2). Hunter color parameters and sensory parameters (color, texture, and appearance) of the fillet did not change (6–18 W/m^2), while flavor and overall acceptability decreased at the dose of 6 W/m^2.

45.3.6 Dairy

A 6 log reduction of the initial microbial population was achieved using 100 kJ/m^2 of pulsed UV when goat milk was inoculated with *E. coli* [46]. Samples irradiated with 50 and 100 kJ/m^2 doses exhibited aromatic changes as compared with the non-irradiated, while no significant differences in the physical or compositional parameters were observed. The inactivation of *E. coli* O157:H7 in bovine milk was higher when exposed to 254 nm UV as compared to 222 and 282 nm at the same fluence of 50, 100, and 200 J/m^2 [47]. The reactivation was increased as the incubation time and temperature (5, 20, 37°C) increased regardless of the UV light sources (i.e. under dark incubation phases), while the reactivation ratios

were lower than those of non-UV-treated cells regardless of the incubation temperature. The lowest reactivation ratios were observed after milk exposure to the UV light at 254 nm and 4°C as compared to 222 and 282 nm exposure.

UV and heat pasteurization treatments caused loss of vitamin C in milk. In the cases of cow and goat milk, pasteurization did not show any significant effect on vitamin B_2. However, UV light treatment decreased the amount of vitamin B_2 after several passes of milk through the UV system. UV light sensitivities of vitamins were C > E > A > B_2, and sensitivity depended on the number of passes, and initial levels before UV treatment [28].

The fat oxidation by photochemical action results in off-flavors, such as rancidity, tallowiness, fishiness, cardboard flavor, and oxidized flavor [48]. Coe and Le-Clerc [49] attributed rancidity to the ultraviolet light range of the spectrum. The defects of milk, such as fishiness [50] and cardboard-like flavor [51], butterfat [20], and processed cheese [52] were caused by light oxidation. Thus, packaging materials with the ability to screen UV have been developed for food products. Processed cheese in normal cellophane wax-coated wrappers becomes oxidized within 12 h, and within 48 h the top slice became inedible [48]. This process can be retarded by incorporating a substituted benzophenone within the wax coating normally applied to certain types of cheese wrappers.

Hirsch [53] mentioned that it is accurate to say that the meaty portion of bacon is subject to fading when exposed to UV light. Fade can be reduced appreciably through vacuum packaging and a UV barrier on the packaging material. A good vacuum-packaging operation will deliver a bacon package with no less than 28 in of vacuum. By incorporating polyvinylidine chloride (PVDC) into the packaging material both oxygen and UV light are screened, and the product survives a considerably longer period of time without fade. Generally, retinoids are very susceptible to oxidation because of their alkyl chains with highly conjugated double bonds [54]. Shimoyamada et al. [54] found that retinol and retinoic acid bound to β-lactoglobulin were less susceptible to light-induced oxidation by UV light irradiation than those which were free or bound to bovine serum albumin. They found a different mechanism of protection against light-induced oxidation compared to enzymatic oxidation. Jung et al. [55] found that the addition of ascorbic acid greatly inhibited the light-induced reduction of all-*trans*-retinyl palmitate and 13-*cis* isomer in skim milks. Ascorbic acid also greatly increased the formation of 9-*cis*-retinyl palmitate in skim milk during light storage.

45.4 UV IN SANITATION

The disinfection (treatment time: 3–60 min) effectiveness of lettuce, tomatoes, and carrots was compared when treated with chlorine (50, 100, and 200 ppm), citric acid (0.5, 1, and 1.5%), UV-C (0.65 and 16 kJ/m^2), and ozone (5 ppm) [56]. Citric acid did not prove effective for the inactivation of *E. coli* ATCC 11775, while UV-C was effective when fluence was higher (i.e. more effective in the smooth surface of tomato, 2.7 log reduction), and ozone was able to inactivate bacteria in tomatoes (2.2 log reduction) after only 3 min. In all treatments, carrots and lettuce showed lower inactivation because of their porous and rough surfaces. UV-C produced most effects on the color, therefore sensory properties need to be considered in addition to the effectiveness of microbial disinfection.

UV rays (non-ionizing radiation) have been used extensively in the disinfection of equipment, glassware, and air by industries for many years [57]. The bactericidal effect of ultraviolet light is widely used for sanitation purposes. It is particularly effective in destroying airborne organisms and consequently has become an important sanitary aid to in-plant installations. It may eliminate detrimental contamination and keep away objectionable invaders. Cerny [58] found that high-intensity UV irradiation may be used in the sterilization of packing materials for aseptic packaging. The penetrating power of ultraviolet rays is very low, so that lethal action is confined to organisms on or near the surface of irradiated materials. Aerial disinfection is severely limited by the presence of dust particles in the atmosphere. Several different UV lamps are available commercially for food industry applications for processing or disinfection.

45.5 PHOTO REACTIVATION

If microorganisms are treated with various dyes (erythrosine, for example), they may become sensitive to damage by visible light. This effect is known as *photoreactivation*. Some food ingredients could possibly induce the same reaction. Such dyes are said to possess photodynamic action [1]. Spores may occasionally fail to show photoreactivation when inactivated with UV light, whereas the corresponding vegetative cells sometimes show photoreactivation. The simplest explanation of these data is to assume that radiation damages genetic material in the spore, and that certain bacteria may produce diploid spores as a result of specific disruptions. Such bacterial spores exhibit two types of radiation inactivation curves: *B. subtilis*, *Bacillus brevis*, and *Bacillus mesentericus* are inactivated in a single-hit fashion, whereas *Bacillus megaterium*, *Bacillus cereus*, and *Bacillus mycoides* are affected by a multiple-hit approach. In all cases, there is no effect on spore survival if the post-irradiation medium is changed from yeast extract to a purely chemically defined medium [1].

When surface microbial contamination is the major cause of spoilage in the case of selected seafood, the application of intense, short pulses of incoherent, continuous, broad-spectrum light can be used to increase the shelf life. The extension is achieved through two processes: (i) by the destruction of spoilage-causing microorganisms, and (ii) by the inactivation of enzymes. This destruction or inactivation is obtained through a complex photothermal and photochemical mechanism mediated by the use of wavelengths less than 300 nm. The pulsed light waves transfer thermal energy to a thin surface layer without raising the interior temperature of the product [2]. Colby and Flick [2] concluded that the increased efficiency is possible to achieve by the use of dyes or other chemical compounds that selectively bind to either

microorganisms or enzymes thereby increasing their susceptibility to the pulsed electromagnetic waves.

Photo-induced (light-induced) off-flavors also result from lipid oxidation and protein degradation. Linoleic acid is highly susceptible to photo-oxidation, and can lead to the formation of high levels of hexanal. Cabbagy, burnt-protein, and burnt-feather notes are associated with protein degradation. Amino acid degradation is catalyzed by riboflavin photo-oxidation and results in thiols, sulfides, disulfides, and 3-methylthiopropance of riboflavin, and oxygen generates methional, which further degrades to methanethiol, dimethyl sulfide, and dimethyldisulfide, all of which contribute to a cabbage or burnt-feather type aroma. Maillard reaction heterocyclic intermediates 2-ethylpyrrole, 2-ethylfuran, and 2,4,5-trimethyloxazole decrease in concentration as a result of photo-oxidation in the presence of chlorophyll [59, 60].

The pH of fructose solution decreased by 5.29% upon exposure to high-intensity pulsed UV light due to the photolysis of fructose when exposed to UV light at 254 nm [61]. UV light (254 nm) exposure of fruit juice with fructose can form furan [62] and accelerate the degradation rates of patulin and ascorbic acid of apple juice [63, 64]. Fructose can be used as a photosensitizer for the accelerated photo-degradation of chlorpyrifos (i.e. insecticide) and diuron (i.e. herbicide) [65]. The reactive oxygen species (ROS), primary species (such as hydroxyalkyl, and hydroxyalkyl acyl radicals), secondary species (hydroxyl, peroxyl, superoxide radicals, and hydrogen peroxide), singlet oxygen, acidic photolysis was generated in the fructose solution when exposed to 254 nm UV light [65–67].

45.6 CONCLUSION

UV rays are extensively used in industry for disinfecting equipment, glassware, air, and water. A low dose of UV usually extends the shelf life of foods without serious changes in quality. However, at high doses marked changes, such as flavor, odor, fat oxidation, and loss of vitamins, are observed before satisfactory sterilization. Mild doses of UV are successfully applied in different food products, such as juice, fresh and minimally processed fruits and vegetables, fresh and dried fish, and dairy products. Photo-reactivation could occur in many food components, such as fructose and color pigments, and could enhance the effectiveness of UV treatment. However, this treatment can cause positive or negative effects on food products, and thus needs to be applied carefully.

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46 High-Intensity Pulsed Light for Food Preservation

M. L. Bhavya and H. Umesh Hebbar

CONTENTS

46.1 INTRODUCTION

Urbanization has changed the lifestyles of people and their priorities towards the consumption of processed foods, and the trend is on the rise. Thermal processing of foods ensures prolonged shelf-life and food safety; however, it also contributes to undesirable changes in the food matrix affecting the functionality of foods and their flavor, colors, vitamins, and textural softening [1]. In recent years, consumers' demand for minimally processed foods with nutrients has increased drastically. Simple, cost-effective, and energy-efficient novel alternative techniques are gaining importance in meeting consumers' demands. Many innovative technologies have been developed to inactivate microorganisms, such as pulsed electric field, high hydrostatic pressure, ultrasound, dense phase carbon dioxide, cold plasma, ozone, and pulsed light (PL), and these are gaining popularity in the food-processing sector. These novel technologies have potential in preserving the nutritional and delicate sensory qualities of food and hence can be used for the minimal processing of foods [2]. Among these non-thermal technologies, one of the emerging technologies is light-based processing, which not only reduces the microbial load but also retains the nutrient quality of solid and liquid foods. This novel technology is being employed to decontaminate foods, packaging material, medical devices, and processing equipment related to the food, medical, and pharmaceutical sectors [3–7].

46.1.1 Pulsed Light Technology

PL technology is a novel non-thermal processing, where decontamination of foods is achieved by the application of high-intensity light pulses within a short duration of time. The term PL has been recognized since the year 1980 and was first implemented by the FDA for processing of food in the year 1996 [8]. The PL constitutes a wide wavelength range

of 180–1100 nm, which includes ultraviolet (UV): 180–400 nm, visible (VIS): 400–700 nm, and the near-infrared region (IR): 700–1100 nm [4, 9, 10]. Irradiation with PL of high peak power is used to deliver a spectrum 20,000 times more intense than sunlight at the earth's surface [4, 11]. One of the major benefits of PL is its short-duration energy exposure as compared to the static UV treatment [12, 13]. The foremost advantages of PL are (i) its substantial microbial reduction in very short treatment time, (ii) absence of chemical compounds, (iii) its low impact on the environment [7, 14], (iv) its operation at relatively low costs, and (v) it generally does not affect the characteristics of food matrices [15]. PL has potential applications in food processing by reducing risk to public health from foodborne pathogens, extending the shelf life of the food product, and improving economics during food distribution [16]. Although, PL processing is considered "non-thermal," it has the drawback of heating the sample if prolonged treatment is used. Significant increase in temperature is due to longer pulsed ultraviolet (PUV), and it has an extra thermal effect on microbial reductions [17, 18].

46.1.2 Terminology

The following terminology and units are frequently used in PL treatment [9]: pulse width (i.e. time interval during which energy is delivered, s), fluence (i.e. energy received from the lamp by the sample per unit area during the treatment, expressed as J/m^2), energy dose (i.e. used synonymously in place of fluence), peak power (i.e. pulse energy divided by the pulse duration and measured in Watt, W), fluence rate (i.e. energy received from the lamp by the sample, W/m^2), and exposure time (i.e. duration of the treatment, s).

46.2 PULSED LIGHT SYSTEMS

Batch and continuous systems are used for microbial load reduction in various food products. Most of the batch systems have almost similar constructions with different types of light sources and product cooling mechanisms. Continuous-flow systems are reported to be better suited, as they provide good mixing and enhance the feed handling rate. A few reports on batch and continuous systems for PL processing are discussed below.

46.2.1 Batch Systems

MacGregor et al. [19] used a bench-top PL system for the inactivation of food-related pathogenic bacteria. The chamber was designed to keep the samples at an inclination of 45° to receive equivalent doses of light energy from a xenon lamp, which is placed in the chamber. At the upper level of PL (512 μs), reductions of 6 and 7 log were observed for *Listeria monocytogenes* and *Escherichia coli* respectively. Fine and Gervais [20] used a 3-liter fluidized-bed system with adjustable air nozzles and compressed air to allow the tangential blowing of fluidized food powders. This system uses high intensity with broad spectra white light, whereas a flash lamp was surrounded by a quartz jacket with water circulation to limit lamp overheating and a reflecting cylinder. Sharma and Demirci [21] and Ozer and Demirci [16] decontaminated alfalfa seeds and salmon fish fillets using batch systems. Similarly, Bialka and Demirci [22] also constructed a laboratory-scale, batch PL system for the sterilization of blueberries with a slight alteration in the set-up consisting of a quartz window and a cooling blower. Choi et al. [23] designed a PL system for the sterilization of infant foods that consists of a water bath as a cooling device to dissipate the heat produced during the discharge of PL and an oscilloscope to observe the exponential decay pulse. Luksiene et al. [24] fabricated a high-power PL system with a xenon flash lamp as the light source. Paskeviciute et al. [25] and Luksiene et al. [26] used this unit for chicken, vegetable, and fruits sterilization. Cheigh et al. [27] constructed a lab-scale PL system comprising a xenon lamp used to produce intense pulsed light (IPL) for inactivating *L. monocytogenes* on solid medium and seafoods (Figure 46.1). Hwang et al. [28] designed a batch IPL treatment unit for the microbial inactivation of various liquid samples (mineral water, isotonic beverage, fruit juices, carbonated beverages, milk). The samples were exposed to a xenon lamp placed in a chamber, and to prevent the overheating of samples during processing, an air cooling system was provided on either side of the lamp.

46.2.2 Continuous-Flow Systems

Krishnamurthy et al. [29] conducted PUV treatment in the continuous flow-through system for inactivating *Staphylococcus aureus* in milk. Milk was pumped through a narrow quartz tube exposed to PUV, placed inside a chamber. V-groove reflectors made up of a polished metal surface were provided to enhance the energy absorption by milk. The authors concluded that effective inactivation of *S. aureus* (up to 7.27 log) in milk can be carried out in a continuous-flow system. Pataro

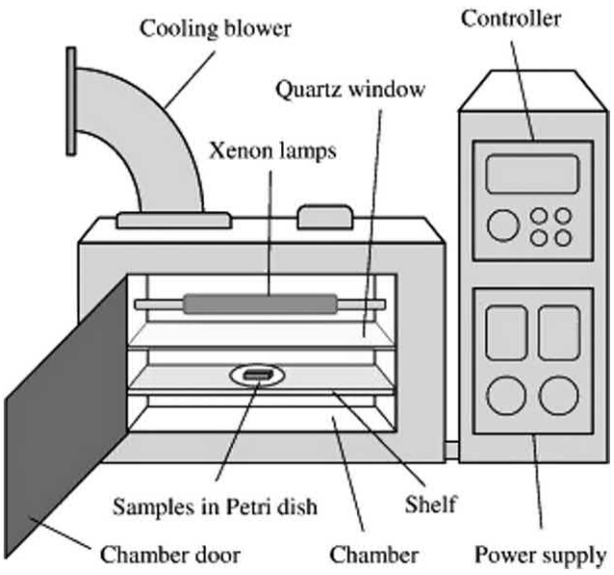

FIGURE 46.1 Schematic diagram of the intense pulsed light (IPL) system. (From Cheigh et al. [27].)

et al. [30] reported their work on bacterial (*Listeria innocua* and *E. coli*) inactivation of fruit juices (apple and orange) in a laboratory-scale continuous-flow PL system. Pre-cooled juice was pumped through two consecutive quartz tubes exposed to xenon lamps (pulse duration of 360 μs), and the heating of the product was controlled through a water–ethylene glycol solution refrigeration system. The present system was able to inactive up to 4 log under optimized conditions of processing. The authors suggested that proper designing of the system is essential to minimize the temperature build-up and to improve treatment homogeneity. Caminiti et al. [31] developed a continuous-flow system to study the impact of a select combination of non-thermal processing technologies, including light-based technology, on the quality of an apple and cranberry juice blend (Figure 46.2). The juice was pumped through two quartz glass tubes and exposed to PUV (360 μs) and cooling assembly prevented heating of the product while flowing through tubes. The unit was able to handle nearly 20.8 ml of juice per min and the authors did not observe any significant difference in appearance, sweetness, or acidity of the juice processed by PUV.

A pilot-scale flow-through system was designed by Chaine et al. [12] for the decontamination of sugar syrup. A xenon flash lamp was fitted inside a stainless-steel tube, using a quartz sleeve, and the sugar syrup was passed through a narrow gap between the steel tube and quartz sleeve. At an optimized flow rate of 5.3 ml/s, at least a 3 log reduction of various microbes was achieved. Based on the result, the authors recommended the use of several parallel modules to increase the volume of feed. Muñoz et al. [32] used a continuous PL system consisting of two quartz tubes for the inactivation of microbes in pre-cooled juice, where a xenon flash lamp with a pulse generator was used for the generation of PL (Figure 46.3). The authors inferred that a combination of PL and thermosonication (prior to PL) is more effective than the individual application. In another study, a similar system was developed to treat apple juice, in which the authors compared the effect of PL with and without the combination with ultrasound (US) treatment on different microbes [33]. Juice was pumped through two consecutive quartz tubes exposed to a xenon lamp and recirculated for the desired degree of exposure, and the product temperature was maintained through an

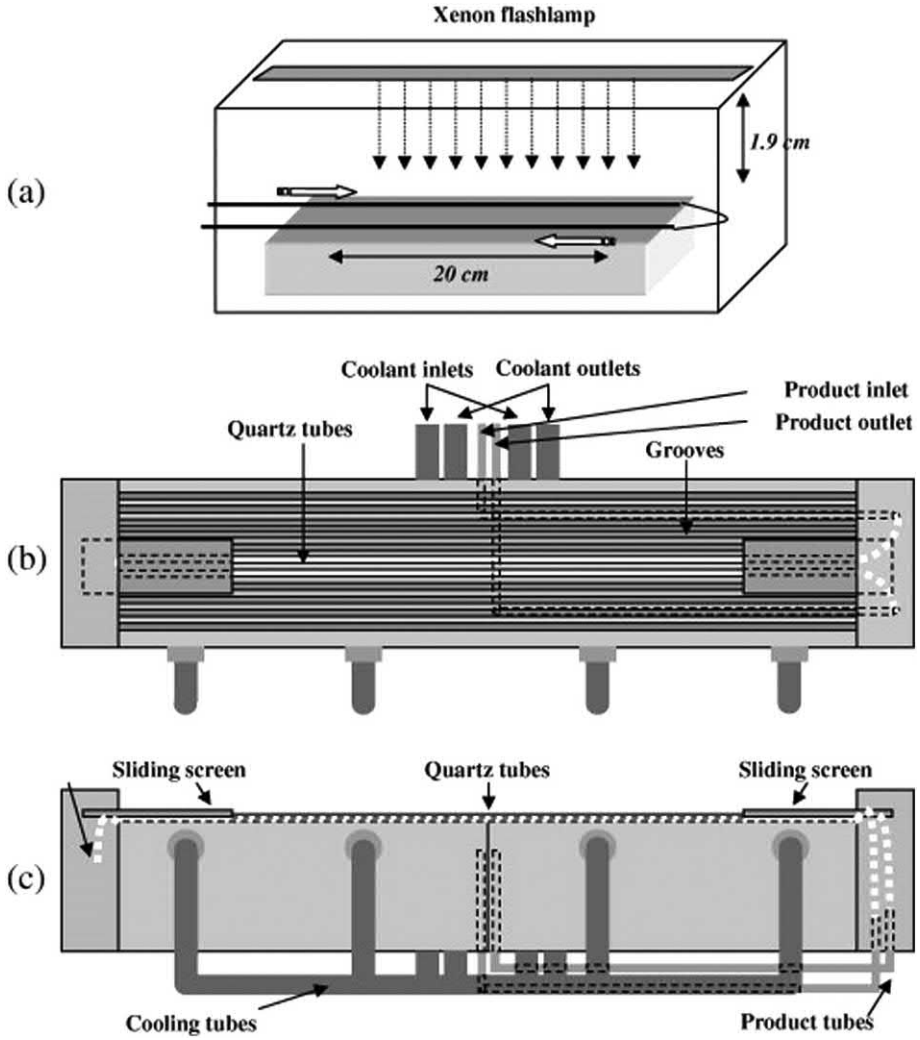

FIGURE 46.2 (a) Schematic diagram of the high-intensity light pulse (HILP) processing system; (b) top view of the continuous-flow HILP chamber; (c) side view of the continuous-flow HILP chamber. (From`Caminiti et al. [31].)

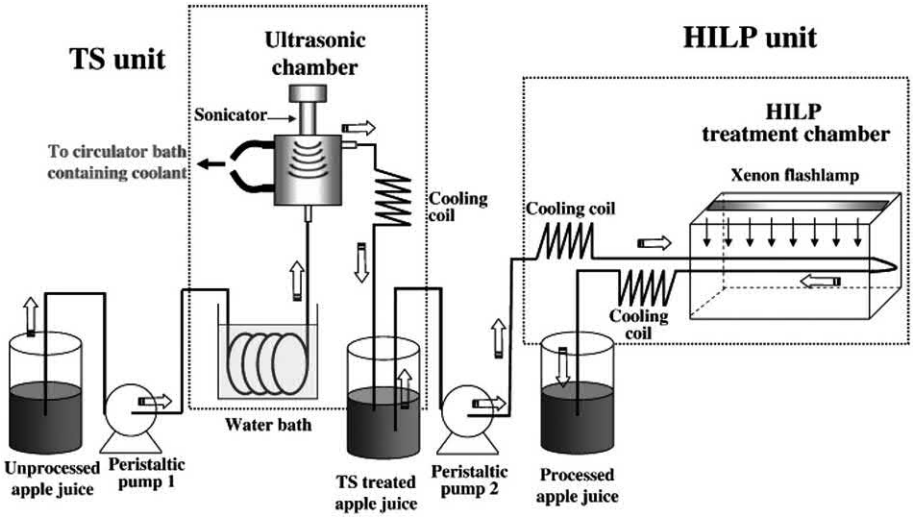

FIGURE 46.3 Schematic diagram of a thermosonication (TS)/high-intensity light pulse (HILP) processing system for reconstituted apple juice. (From Muñoz et al. [32].)

external cooling system. The results have shown that a continuous PL system can be effectively used in combination with other techniques. Yi et al. [34] developed a pilot-scale continuous-flow IPL system (Figure 46.4) to inactivate bacteria and viruses in untreated groundwater. A cylindrical treatment chamber (0.93 m height × 0.31 m diameter) was used to circulate water in the chamber using a pump, and the treatment time was set by controlling the flow rate. Multiple cylindrical xenon flash lamps (15 nos.) with emission spectra ranging from UV to infrared were used in the study. They reported a 2.91–4.70 log reduction of various microbes at different processing times and energy doses. At the optimized rate, the developed unit was estimated to handle 720 L/h of water, and it was suggested that it could be used in the food industry for the treatment of water.

PL-based systems are being manufactured to suit commercial-scale operations. The pioneering company to produce PL equipment for application in water purification and virus inactivation was Purepulse Technologies Inc. (San Diego, California), a subsidiary of Xenon Corp., which commercialized the PureBright™ system [11]. As per literature reports, there are three major commercial companies, namely, SteriBeam Systems from Germany, Xenon Corporation from the United States, and Claranor from France, producing PL-based disinfection systems. The details of various systems used for PL processing are presented in Tables 46.1 and 46.2.

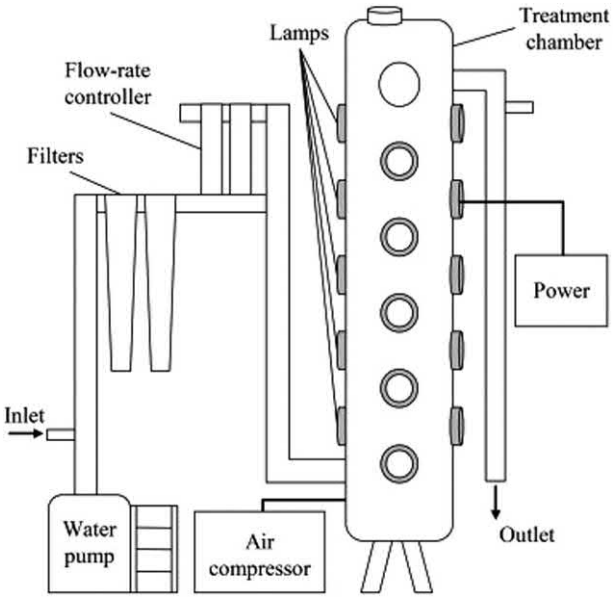

FIGURE 46.4 Pilot-scale continuous-flow PL system. (From Yi et al. [34].)

46.3 PULSED LIGHT FOR MICROBIAL INACTIVATION

46.3.1 Effect on Microorganisms: Bacteria, Fungi, Viruses, and Spores

The susceptibility trend is reported to be Gram-negative bacteria > Gram-positive bacteria > bacterial spores > fungal spores > viruses [35–38]. Gram-positive bacteria, *L. innocua*, are more resistant to PL when compared to Gram-negative bacteria, *E. coli*, which may be due to the distinguished compositional/structural variation in the cell walls of these bacteria [12, 39–41]. In contrast, Palgan et al. [10] found that *E. coli* (4.67 log reduction) was more resistant than *L. innocua* (5.13 log reduction) when inoculated in maximum recovery diluent solution. According to Luksiene et al. [26] and Hilton et al. [42], *Bacillus* was more susceptible than mesophilic bacteria, and *L. innocua* was more resistant than *Pseudomonas fluorescens* to PL at low temperatures and low fluence levels. Higher PL resistance shown by *Listeria* spp. (<1 log reduction), as compared to *Pseudomonas phosphoreum* (5 log reduction) and *Serratia liquefacans* (3.9 log reduction), could be related to the existence of photoreactive substances and protective

TABLE 46.1
PL Processing of Fruits and Vegetables

Product	Processing System/Conditions	Microbial Safety Parameters	Reference
Apple juice	Laboratory-scale continuous-flow PL unit that produces 3 pulses/s with approx. 1.21 J/cm^2/pulse at 1.9 cm from the quartz window surface (SteriPulse-XL 3000)	4 and 2.98 log reduction of *E. coli* and *L. innocua* at 4 J/cm^2	Pataro et al. [30]
Orange juice		2.90 and 0.93 log reduction of *E. coli* and *L. innocua* at 4 J/cm^2	
Commercial apple juice	Continuous-flow PL system (RS-3000B SteriPulse-XL)	4.2, 1.8, and 3.1 log reduction of *S. enteritidis*, *S. cerevisiae*, and *E. coli* at 0.73 J/cm^2	Ferrario and Guerrero [33]
Cucumber slices	Bench-top PL system (RS-3000C SteriPulse- XL)	0.6, 2.2, and 2.8 log reduction of *E. coli* at 4, 8, and 12 J/cm^2 respectively	Tastan et al. [127]
Fresh-cut tomatoes	Automatic flash lamp system (SteriBeam Xe-Matic-2L-A)	4.2 and 3.8 log reduction of mold and yeast count at 6 and 8 J/cm^2 respectively	Valdivia-Najar et al. [82]
Fresh-cut avocados	Automatic laboratory flash lamp system (SteriBeam XeMaticA-2L)	1.05, 0.85, and 1.2 log reduction of aerobic mesophilic count at 3.6, 6, and 14 J/cm^2 respectively	Aguiló-Aguayo et al. [108]
Strawberries	Bench-top PL system (SteriPulse- XL RS-3000)	1.4–2.4 log reduction of *E. coli* O157:H7 at 5.6–63.2 J/cm^2 of WPL treatment	Huang et al. [91]
Raspberries		1.6–4.5 log reduction of *E. coli* O157:H7 at 4.8–53.9 J/cm^2 of WPL treatment	
Mango slices	PL system- XeMaticA-2L (SteriBeam)	3.5 and 2.07 log reduction of *L. innocua*, and yeast and mold count at 8 J/cm^2 respectively	Salinas-Roca et al. [94]
Apple juice	Steri-Pulse XL-3000 PL sterilization system	1.93 and ≥4.7 log reduction of *L. innocua* and *E. coli* at 8 s PL treatment respectively	Palgan et al. [10]
Apple juice	RS-3000B Steripulse-XL system	1.0, 1.6, 2.1, and 2.4 log reduction of *S. cerevisiae*, *L. innocua*, *E. coli*, and *S. enteritidis* respectively at 71.6 J/cm^2	Ferrario et al. [54]
Fresh-cut avocado	XeMaticA-2L system (SteriBeam)	2.97 and 3.33 log reduction of *L. innocua* and *E. coli* respectively at 12 J/cm^2	Ramos-Villarroel et al. [41]
Apple juice	RS-3000B Steripulse- XL system	3.9 and 1.0–2.0 log reduction of *S. cerevisiae* KE 162 cells in commercial and natural apple juice respectively at 60 s PL	Ferrario and Guerrero [63]
Mung bean sprouts	PL chamber with three xenon tube reflector (Claranor)	1.91, 1.55, and 1.65 log reduction of *E. coli*, *L. innocua*, and natural microbiota respectively	Kramer et al. [56]
Endive salad		2.34, 2.54, and 2.46 log reduction of *E. coli*, *L. innocua*, and natural microbiota respectively	
Raspberries	Batch-fed PL system (SteriPulse-XL 3000)	0.4–3.9 log reduction of *E. coli* and 0.3–3.4 log reduction of *Salmonella* at 2.9–72 J/cm^2 respectively	Bialka and Demirci [17]
Plums	PL system (laboratory construction)	1.5 and 1.4 log reduction of mesophiles and *B. cereus* respectively	Luksiene et al. [26]
Tomatoes		1.2 and 1.5 log reduction of mesophiles and *B. cereus* respectively	
Cauliflowers		1.1 and 1.3 log reduction of mesophiles and *B. cereus* respectively	
Sweet peppers		1.3 and 1.8 log reduction of mesophiles and *B. cereus* respectively	
Strawberries		1.1 and 1.5 log reduction of mesophiles and *B. cereus* respectively	
Apple cider	RS-3000C SteriPulse system	1.49 log reduction of *E. coli* ATCC 25922	Sauer and Moraru [13]
Apple juice		1.62 log reduction of *E. coli* ATCC 25922	

(*Continued*)

TABLE 46.1 (CONTINUED)
PL Processing of Fruits and Vegetables

Product	Processing System/Conditions	Microbial Safety Parameters	Reference
Apple juice	SteriBeam XeMaticA-2L system	1.27–3.76 log reduction of *P. expansum* at 4–32 J/cm^2 respectively	Maftei et al. [84]
Commercial apple juice	RS-3000B SteriPulse-XL system	3.0 and 4.4 log reduction of *A. acidoterrestris* spores and *S. cerevisiae* cells at 71.6 J/cm^2	Ferrario et al. [119]
Apple juice	PL mobile decontamination system (Claranor)	3.0 and 2.7 log reduction of *L. brevis* and *L. monocytogenes* at 17.5 J/cm^2	Ignat et al. [87]
Raspberries	RS-3000C SteriPulse system	4.5 log reduction of *Salmonella* at 28.2 J/cm^2	Xu and Wu [65]
Fresh-cut mushrooms	SteriBeam XeMaticA-2L system	3.03 and 2.66 log reduction of *E. coli* and *L. innocua* respectively at 12 J/cm^2	Ramos-Villarroel et al. [40]

compounds, which contribute to the antimicrobial efficiency of PL [43]. PL caused reduction of *E. coli*, *L. monocytogenes*, *Salmonella typhimurium*, and *Vibrio parahaemolyticus* in agar by 5.8, 6.1, 6.0, and 5.4 log units respectively at 0.525 J/cm^2 [44]. A single PL (0.6 J/cm^2) was sufficient to reduce *Bacillus subtilis* by 8.7 log units in suspension [45]. McLeod et al. [46] observed a 5–7 log reduction of microbes tested on agar in petriplates, and found higher resistance of *L. monocytogenes* (4 log reduction) to PUV treatment at low fluence of 1.25 J/cm^2. Ganan et al. [47] reported that *S.* Typhimurium

TABLE 46.2
PL Processing of Meat, Marine, and Poultry Products

Product	Processing System/Conditions	Microbial Safety	Reference
Egg pasta	PL system (Claranor) with dose from 0.13 to 1.75 J/cm^2/pulse and experiment was carried out at 25°C	2.5 log reduction of *S. enterica* 9898 DSMZ at 0.7 J/cm^2	Manzocco et al. [90]
Skinless chicken fillets	Semiautomated intense PUV system (SteriBeam system)	Reduction range: 0.9–2.4 log for *S. enteritidis*, 1.1–2.0 log for *L. monocytogenes*, 1.3–3.0 log for *S. aureus*, 1.7–3.0 log for *Pseudomonas spp.*, 1.3–3.0 log for *B. thermospacta*, and 1.3–2.8 log for *E. coli* when treated at 1.25–18 J/cm^2	McLeod et al. [46]
Ham slices	Steribeam SBS-XeMatic-2L-A device (SteriBeam system)	1.78 log reduction of *L. monocytogenes* at 8.4 J/cm^2	Hierro et al. [97]
Bologna slices		1.11 log reduction of *L. monocytogenes* at 8.4 J/cm^2	
Eggs	Steribeam SBS-XeMatic-2L-A device (SteriBeam system)	0.14–2.49 log reduction of *S. enteritidis* at 2–12 J/cm^2 respectively	Hierro et al. [99]
Shrimp	Lab-scale PL system	2, 2.2, and 2.4 log reduction of *L. monocytogenes* at 0.7, 6.3, and 12.1 J/cm^2 respectively	Cheigh et al. [27]
Salmon		1.8, 1.9, and 2.1 log reduction of *L. monocytogenes* at 0.7, 6.3, and 12.1 J/cm^2 respectively	
Flatfish		1.6, 1.7, and 1.9 log reduction of *L. monocytogenes* at 0.7, 6.3, and 12.1 J/cm^2 respectively	
Eggs	Steribeam SBS-XeMatic-2L-A device (SteriBeam system)	3.3 and 5 log reduction of *Salmonella* at 0.35 and 2.1 J/cm^2	Lasagabaster et al. [100]
Beef *carpaccio*	Steribeam SBS-XeMatic-2L-A device (SteriBeam system)	1.2, 1.0, and 0.8 log reduction of *E. coli*, *S. typhimurium*, and *L. monocytogenes* respectively at 11.9 J/cm^2	Hierro et al. [44]
Tuna *carpaccio*		1.0 and 0.7 log reduction of *V. parahaemolyticus* and *L. monocytogenes* respectively at 11.9 J/cm^2	
Egg shells	SteriPulse XL-3000 PUV system	2.0 and 7.7 log reduction of *S. enteritidis* at 1.2 and 35.3 J/cm^2 respectively	Keklik et al. [116]
RTE Sausages	RS-3000C SteriPulse system	1.37 log reduction of *L. innocua* at 9.4 J/cm^2	Uesugi and Moraru [14]
Salchichon	Steribeam SBS-XeMatic-2L-A device (SteriBeam system)	1.81 and 1.48 log reduction of *L. monocytogenes* and *S. typhimurium* respectively	Ganan et al. [47]
Dry cured loin		1.61 and 1.73 log reduction of *L. monocytogenes* and *S. typhimurium* respectively	

is more resistant than *L. monocytogenes*. The effectiveness PL treatment is independent of temperature for *E. coli* and *P. fluorescens* in clear liquid substrates within the temperature range of 5 to 40°C, but a modest synergistic effect of temperature and PL was observed for *L. innocua* at 50°C [42]. IPL sensitivity in microorganisms may be related to differences in bacterial cell wall composition due to their protective and effective mechanisms to repair against the damage [48]. Gomez-Lopez et al. [5] demonstrated that the degree of inactivation of *L. monocytogenes* achieved by PL treatment was independent of the successive flashing, i.e. the microbe did not show any resistance to PL treatment. PL induced sublethal injury in *Saccharomyces cerevisiae* cells at low doses up to 12 J/cm^2, showing that the recovery of cells is dependent on the medium [49]. This treatment causes sublethal damage, which makes cells more sensitive to stress in subsequent stages, such as storage at low temperatures [50]. In contrast, PL did not induce any sublethal damage to the bacterial cells, such as *L. monocytogenes*, *E. coli*, *V. parahaemolyticus*, and *S.* Typhimurium [44]. *Salmonella* and *Listeria* were unable to repair the cell damage induced by PL through the photoreactivation mechanism [25].

The PUV inactivated *B. subtilis* spores in aqueous solution by >5 log cycles at a fluence of 60 mJ/cm^2 [51] and 5 log reduction on agar and treated at 1.24 J/cm^2 PL [37]. Spores are more resistant to PL than vegetative cells [52]. For a cell density of 10^7 cells/ml, fluence of 0.5 J/cm^2 induced >5 and 3 log reductions of vegetative cells and spores of *B. subtilis*, respectively. A 7.9 log reduction of *B. subtilis* spores in suspension was observed at 12 J/cm^2 PL application, and lower total fluences (0.3–5.5 J/cm^2) had no influence on germination, but retarded vegetative growth [53]. Ferrario et al. [54] found that *S. cerevisiae* was more resistant to PL treatment than *E. coli*, *L. innocua*, and *Salmonella* Enteritidis in PL-flashed apple juice. In contrast, Nicorescu et al. [45] have reported that bacteria is more resistant than yeast for PL treatment. *Aspergillus niger* was the more resistant microorganism to UV-C compared to *B. subtilis*, *Geobacillus stearothermophilus*, *Alicyclobacillus acidoterrestris*, and *S. cerevisiae* [12]. The vegetative cells are more sensitive to PL than spores [5]. Marquenie et al. [55] observed that conidia of *Botrytis cinerea* and *Monilinea fructigena* was reduced by 3 and 4 log units, respectively, and single light pulse did not lead to the development of any resistance to PL treatment. Inactivation of *B. cinerea* conidia was higher on nutrient agar as compared to that in suspension [55]. The PL-treated bacterial populations were decreased by 8 log units and fungal counts by 4.5 log units, after 1000 light pulses of the higher UV intensity light [35]. *Aspergillus niger* was more resistant in spore form as compared with *Fusarium culmorum* to PL, and this resistance can be attributed to UV absorbance associated with the dark pigment present in *A. niger* [35]. When UV-C and PL were applied sequentially, regardless of the fluence or duration of treatment, inactivation of *B. cinerea* conidia was increased and the maximal reduction was obtained at the highest doses (0.10 J/cm^2 and 120 s). Combined thermal treatment (45°C for 10 min) and PL (120 s) caused *B. cinerea* conidial reduction of 3.5 log units, an increase of 1 log unit compared to the sum of individual treatments [55]. Viruses were found to be more resistant to PL treatment as compared to bacteria [36]. Murine norovirus (MNV) was inoculated in phosphate-buffered saline solution, and it was observed to reduce by 2.59, 3.77, and 6.69 log units, respectively, when treated (i.e. laboratory-scale IPL system for 30 s) at 0.64, 1.45, and 3.43 J/cm^2, respectively. Inactivation of MNV in water was observed at 3.35 log units after 89 s treatment (4.30 J/cm^2) in a pilot-scale IPL system [34].

46.3.2 Mode of Action

A few studies have reported that VIS or IR region did not contribute any antibacterial effect, while the UV region was proved to be responsible for the inactivation of pathogens [56–58]. Both *Listeria* and *Salmonella* were susceptible to PL (6.5–7.0 log reduction, respectively), and no antibacterial effect was observed when UV light was filtered [25]. Reports are also available indicating that both VIS and IR regions in combination having high peak power also contribute to the destructive effect on microorganisms [4]. The antimicrobial efficacy of UV light on bacteria is due to the absorption of radiation by conjugated carbon–carbon double bonds in proteins and nucleic acids, photochemical dimerization of nucleic acids, and consequent inhibition of transcription and replication, thus resulting in DNA structural changes/cellular inactivation [9, 40, 59]. Sublethal injury caused to bacterial cells by PL processing confirmed that membrane damage is one of the major reasons for bacterial inactivation [30]. Ferrario et al. [49] studied the effect of PL on *S. cerevisiae* through transmission electron microscopy (TEM) and revealed that the loss of viability initiated by PL treatment not only caused membrane damage but also provoked significant disorder in the inner cells.

Cheigh et al. [27] identified the cell damage in *L. monocytogenes*, treated with UV-C and IPL, with the help of TEM analysis. UV-C-treated *Listeria* cells had a similar structure as untreated cells, except for a blurry and indistinct cell wall. In contrast, IPL-treated cells exhibited damage to cell wall structures, cytoplasm shrinkage, and rupture of internal organization leading to leakage of cytoplasmic content and ultimately to cell death [27]. Studies by Takeshita et al. [60] and Krishnamurthy et al. [61] concluded that formation of thymine dimer is the most important reason for inactivation as compared to damage through different mechanisms listed by other researchers. The internal cellular structure collapsing could also contribute to the inactivation of cells by PL and PUV treatment (Figure 46.5). Kramer and Muranyi [62] confirmed that the loss in cell viability by PL application is attributed to oxidative stress associated with DNA damage which results in photochemical inactivation of microbes, apart from its effect on the cell membrane or intracellular enzyme. The TEM studies on *S. cerevisiae* treated with PL revealed that the inactivation process in cells is due to alteration in cell shape, vacuolization, membrane permeabilization, and coagulation of inner cell contents and cell wall [60, 63].

Cheigh et al. [64] reported that IPL application exerted milder photochemical effects on cells than the UV-C

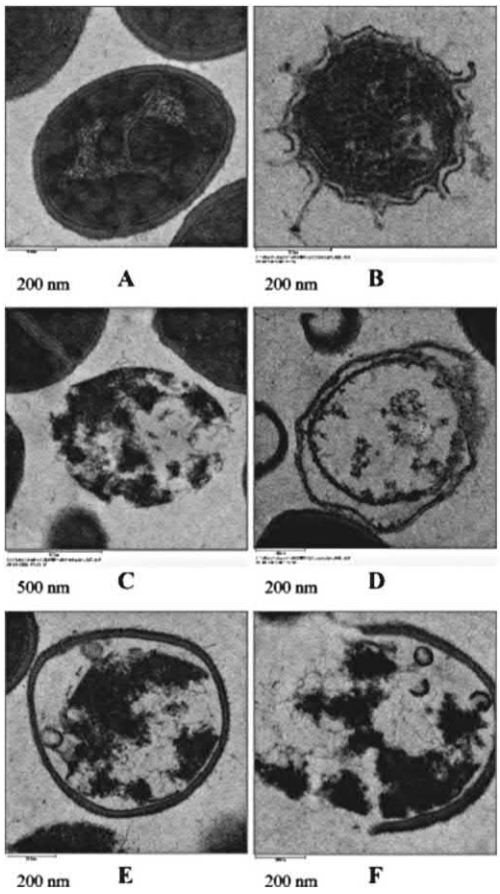

FIGURE 46.5 Evaluation of pulsed UV light (12-ml sample treated for 5 s at 8 cm below quartz window) induced damages in *S. aureus* by TEM: (a) control sample, (b) cell wall rupture, (c) lack of cell wall, (d) cytoplasm shrinkage and cell wall damage, (e) cytoplasm shrinkage and membrane damage, and (f) cell wall damage and cellular content leakage. (From Krishnamurthy et al. [61].)

irradiation, and inactivation of microbes was attributed to other factors, in addition to RNA or DNA damage (Figure 46.6). Nicorescu et al. [45] evaluated the effect of PL on the structural differences in *B. subtilis* inoculated on powdered spices and in suspension with the help of scanning electron microscopy (SEM). The cell membrane was disrupted clearly and formed deep craters in the cell wall after PL treatment on spices. However, in the case of *B. subtilis* treated in suspension, only deformation of the cell wall was observed. The cell wall disruption can be attributed to photothermal stress and germicidal action caused by the PL having a UV component. PL altered the DNA structure by decreasing supercoiling of DNA and further breaking into a single strand, leading to cell death [45]. Treating *E. coli* with PL caused structural changes in membrane integrity leading to flattening of cells, which is attributed to absorption of UV light, that in turn leads to overheating, intercellular water vaporization, and subsequent membrane disruption [65]. The research findings have shown that PL processing involves (Figure 46.7) photothermal/photophysical and photochemical effects, which cause alteration of cell membrane integrity and damage to chromosomal DNA [40, 45, 61, 64, 66].

46.3.3 Inactivation Kinetics

When illustrating microbial inactivation kinetics, the first-order model is widely accepted, especially for thermal treatments. However, it has been shown that the inactivation curves are clearly nonlinear and the use of first-order inactivation kinetics is not suitable for PL inactivation [13, 27, 68–70]. The shape of inactivation curves and lethality of PL treatment depend on the energy dose applied, the absorption properties of the liquid treated, and the types of microorganism [30, 43, 71]. *E. coli* was more sensitive than *L. monocytogenes* to UV-C treatment, and the Weibull model fitted better as compared to the first-order kinetics [17, 72]. The most resistant bacteria, i.e. *L. innocua*, *Pseudomonas*, and *Brochothrix thermosphacta*, displayed typically sigmoidal inactivation curves, with an initial shoulder followed by an exponential loss of cell viability down to the maximum detectable inactivation. However, in the case of the most sensitive species (*Photobacterium phosphoreum*, *Serratia liquefaciens*, and *Shewanella putrefaciens*), no initial shoulder was observed due to the PL treatment (0.053 J/cm^2) [43]. According to Uesugi et al. [68] the nonlinear Weibull model can be used to quantitatively describe microbial inactivation by PL [13]. This model has been used to predict microbial inactivation [13, 33, 42, 46, 56, 68, 73, 74]. The Weibull model (Equation 46.1) is a non-mechanistic model that uses a power function to explain the relationship between inactivation level and dose of the IPL [28, 56, 68, 72]:

$$\mathrm{Log}(N/N_0) = -\alpha * F^{\beta} \tag{46.1}$$

where N is the number of survivors after PL treatment (CFU/g); N_0 is the number of *E. coli* before PL treatment (CFU/g); α is the scale parameter; β is the shape factor, which describes the shape of the curve; and F is the PL fluence (J/cm^2). Inactivation curves exhibited a marked upward concavity, which indicates that the PL process became less efficient for higher doses in the case of commercial and natural juices (Figure 46.8). In commercial apple and natural melon juices, a Weibull-type model was suitable for survival data, except for the *S. cerevisiae* response for which a biphasic model showed better fit [75]. Hilton et al. [42] confirmed that the Weibull model was able to predict the inactivation kinetics of three microbes, namely, *E. coli*, *L. innocua*, and *P. fluorescens* in clear liquids, at sublethal temperatures and fluence below 12 J/cm^2. The Weibull kinetic parameters for PL inactivation in *L. innocua* showed a synergism between temperature and PL inactivation,; a significant temperature dependency of the shape (*n*) and scale (*b*) was observed. However, another study reported that PL inactivation of *E. coli* and *P. fluorescens* was independent of temperature [42]. Ferrario and Guerrero [33] mentioned that the Weibullian model was appropriate for representing survival data, showing higher coefficient of correlation values (95.0% and 99.1%). No differences in the Weibullian-related statistics corresponding to *E. coli* were observed between commercial and natural apple juice

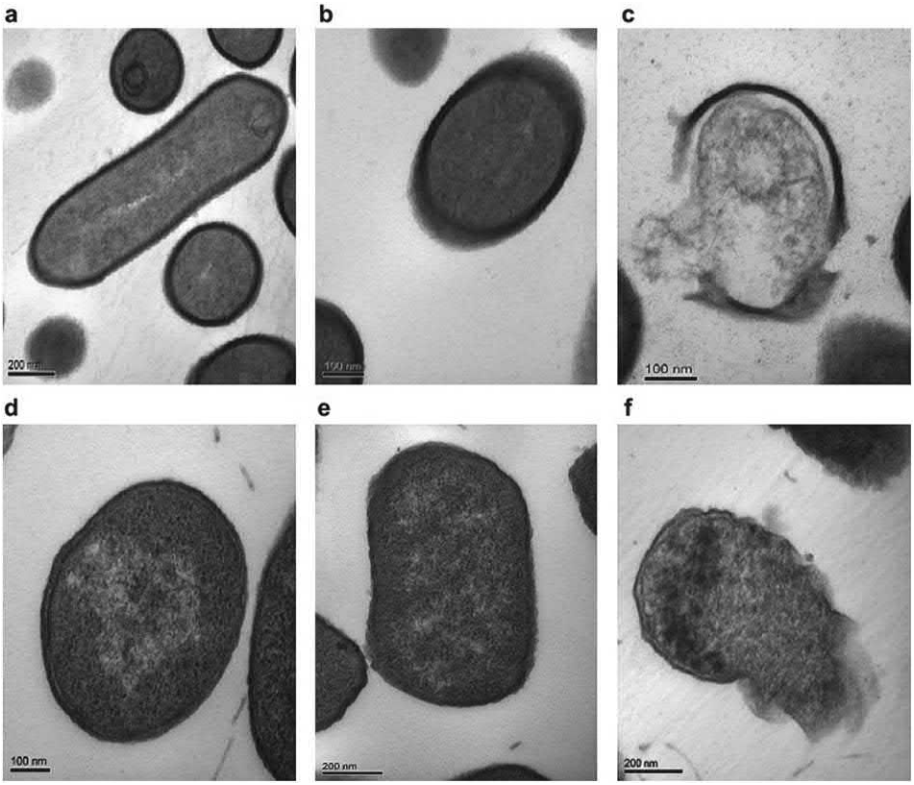

FIGURE 46.6 Transmission electron micrographs of *L. monocytogenes* (a, b, c) and *E. coli* O157:H7 (d, e, f). Images: (a) and (d) untreated control cells, (b) and (e) UVC treatment for 600 s, and (c) and (f) IPL treatment for 180 s at 376 W/m^2. Bars correspond to 100 nm (b–d) and 200 nm (a, e, f). (From Cheigh et al. [64].)

demonstrating that applied treatments exhibited similar effectiveness in both matrixes.

Zenklusen et al. [76] observed that the survival curves of *Aspergillus carbonarius* and *Aspergillus flavus* were non-log linear: the fungal counts steeply reduced (1.2–1.7 log cycles) up to 5–15 s (6–8 J/cm^2) PL treatment. Cheigh et al. [27] used a log-linear model, Weibull model, and modified Weibull model for the inactivation of *L. monocytogenes* (total fluence: 0–17.2 J/cm^2) and the modified Weibull model showed good fitting performance with concave, convex, or linear curves followed by a tailing effect. Common models based on log reduction values for all the species tested on chicken fillets gave a good fit for the majority of the species, but for *L. monocytogenes* exposed to both UV-C and PUV light, reduction was overestimated. It is reported that the Weibull model fits well when the curve is convex, which indicates that exposed cells were destroyed and shows that more resistant cells or those cells sheltered from exposure were left undamaged [46]. The shape of the inactivation curves of *B. subtilis* exposed to PL was dependent on its physiological state. For spores, inactivation curves were typically sigmoidal, showing an initial shoulder followed by an exponential loss of cell viability and a final tailing tendency. The "log-linear with shoulder and tail" model [77] was used to fit the inactivation kinetics of *B. subtilis* spores. In contrast to those found for spores, inactivation curves without a shoulder were shown for vegetative cells. The occurrence of a shoulder or tailing in the inactivation curves could be attributed to differences in cell resistance and/or high absorption in the UV region [52, 75]. Valdivia-Nájar et al., [78] used the modified Gomperz's model on fresh-cut tomato slices during cold storage and mentioned that it provided a good fit to the experimental data with $R^2 > 0.930$. Yi et al. [79] applied the double Weibull model and suggested that this method is suitable for assessing the performance of IPL treatment.

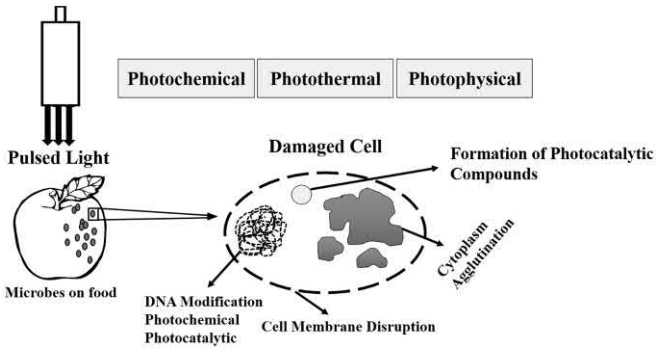

FIGURE 46.7 Mechanism of PL.

46.4 MICROBIAL INACTIVATION BY PULSED LIGHT PROCESSING IN FOODS

46.4.1 Fruits and Vegetables

PL processing has been attempted for various vegetables, fruits, spices, and other products of plant origin for the

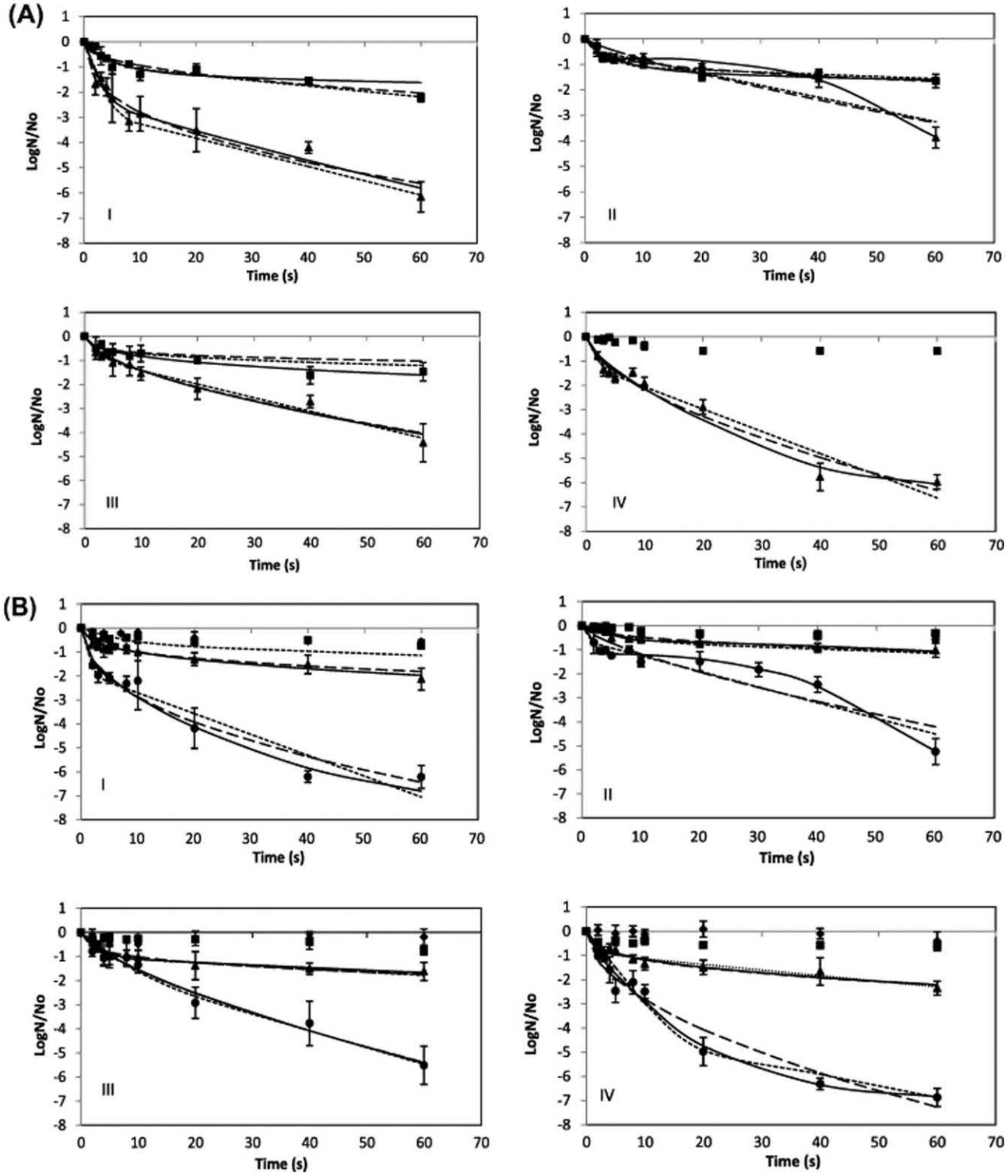

FIGURE 46.8 Experimental survival curves (points) and fitted values derived from Weibull (dashed line), biphasic (dotted line), and Coroller (solid line) models for *E. coli* (I), *S. cerevisiae* (II), *L. innocua* (III), and *S. enteritidis* (IV) in apple (▲); melon (●); orange (■); and strawberry (♦) juices treated with PL. (A) commercial juices; (B) natural juices. (From Ferrario et al. [75].)

decontamination of foodborne pathogens. Literature reports on the inactivation of these pathogens in different fruits, vegetables, and their products are presented in Table 46.1. In order to increase the safety of fruit and vegetable juices, US FDA regulation requires a 5 log reduction process for pathogens [80]. *E. coli* and *Salmonella* populations in blueberries treated with PL decreased with increases in fluence and time. Further, *Salmonella* showed a higher resistance to PL treatment as compared to *E. coli*, when inoculated on the skin of blueberries [18]. The treatment of apple juice with PL reduced *E. coli* counts by 3.1 to 4.9 log CFU/ml, when treated at energy levels of 4.03 to 5.10 J/cm^2 [32]. *E. coli* and *L. innocua* counts were reduced in apple juice treated with PL at 28 J/cm^2, and even after 48 h, there was no subsequent recovery of the cell [10]. With PL application, molds on strawberry fruits were decreased by 16–42% with applied fluence ranging from 2.4 to 47.8 J/cm^2 [81]. Valdivia-Najar et al. [82] observed that when tomato slices were treated with PL (4–8 J/cm^2), psychrophilic bacterial count reduced to 0.7–1.8 log CFU/g from an initial count of 7 log CFU/g.

The efficiency of PL treatment is affected by the type of microbe, microbial population, volume of suspension, and inoculation site [5, 18, 34, 83, 84]. The antimicrobial effect of PL decreased as the population density increased, for both vegetative cells and spores of *B. subtilis* and *G. stearothermophilus* [52]. Decontamination of dip-inoculated was tougher than spot-inoculated samples (by 3.6 log reduction difference at 15 s dry PL) due to penetration of *E. coli* into the open surface structures of the produce [85]. The inactivating effect of PL treatment (16 J/cm^2) against *Penicillium expansum* inoculated in apple juice prominently depended on the microbial population, i.e. 1.30 and 3.2 log reductions for 3×10^5 and 2.3×10^4 CFU/ml, respectively [84]. The inactivation varied based on the growth phase of microbes; for example, reduction was more significant during lag and early stationary phase of growth [5]. The effectiveness of microbial inactivation was influenced by the properties of foods, such as intrinsic transparency, turbidity, absorptivity, reflection coefficient, thickness, color, viscosity, moisture content, the presence of particulate material, and the flow rate of the product [10, 23, 33, 54, 86]. Ferrario et al. [49] stated that both pH and absorbance of the matrix had impact on the PL efficiency, but the absorbance was the most effective factor.

Furthermore, the distance between the sample and light source, treatment time, volume of the sample, sample depth, geometry of the treatment chamber, orientation, and lamp design were the critical factors to be optimized to achieve maximum effectiveness of the PL treatment [9, 29, 65, 87, 88]. The sterilization effects of IPL treatment on *L. monocytogenes* showed significant inactivation compared to UV-C treatment due to the higher penetration depth and emission power of IPL [27]. The inactivation of microbes decreased with the distance when the sample was kept directly below the lamp, but increased at the shelf borders [5]. Efficiency of PL in microbial inactivation is directly dependent on the energy dosage absorbed by microbe, which is affected by the light transmittance through the liquid food [28, 30, 84]. PL has very limited penetration depth in opaque media and is capable of targeting the surface microorganisms [84, 88]. Nearly 5 log and 3.5 log reductions were observed for agar seeded and suspension of *S. aureus* cells, respectively, at 16.8 J/cm^2. In order to achieve a higher microbial inactivation rate in opaque liquids, a high dosage of light is required along with a longer treatment period [28]. The sample depth of suspension cells greatly influences the inactivation level due to the poor penetration capacity of PUV light [86, 88]. Efficiency of PL treatment for the inactivation of *P. expansum* intensely decreased from 3.21 to 1.58 log CFU/ml, when the depth of apple juice was increased from 6 to 10 mm [84]. The inactivation rate of *S. cerevisiae* was higher in a peptone water system than in apple juice possibly due to an increased absorptivity of juice that decreased the effective interaction between microbes and light photons [49]. Another study also showed that PL effectiveness was negatively influenced by the higher absorbance values of liquids in the UV-C region [75].

The properties of the food surface have an impact on the decontamination efficacy of IPL [28, 74]. Studies have proven that physiochemical parameters such as chemical composition, total soluble compounds, pH, and light absorbance (especially due to compounds such as carotenoids) affect the degree of microbial inactivation [49, 78]. The beverages with higher transparency like apple juice, carbonated drink, and plum juice displayed 7 log reductions of *Pseudomonas aeruginosa* with the application of 12.2–24.3 J/cm^2 [28]. While, in the case of milk, grape juice, and coffee, a lower reduction value of 1–1.9 log CFU/ml was observed with a PL dose of 29.2 J/cm^2. Similarly, due to the differences in the transparency of the medium (1 mm thick), lower inactivation levels of *E. coli* and *L. innocua* were also reported by Palgan et al. [10] in milk (1275.2) and orange juice (79.7) when compared to apple juice (5.81) and maximum recovery diluent (0.74) with lower absorption coefficients (ε) (Figure 46.9). PL was more effective in the apple juice system with lower turbidity as compared to orange and strawberry juices, and this signifies that higher turbidity of juices weakens the PL efficiency [54]. One of the important factors that govern the effectiveness of PL is the fluence level applied to the sample [40, 71]. The dose applied and inactivation of microbes are proportional, thus confirming that the PL treatment with higher intensity and pulse number inactivates the microbes to a greater extent [7, 13, 19, 41, 58, 84]. As the distance between the sample and the lamp decreases, the temperature of the product increases drastically [55, 89]. When the spectral range of the PL treatments, particularly the UV component, was altered (normally using filters), the inactivation of *E. coli* and *L. innocua* was also lowered (Ramos-Villarroel et al., 2012). The degree of reduction of *E. coli* and *L. innocua* on fresh-cut avocado flashed with PL containing a UV component (2.74 and 1.35 log CFU/g, respectively) was greater than that treated with PL without UV spectrum (0.83 and 0.68 log CFU/g, respectively) [40]. Other studies have also shown that the UV part of PL contributes more to the inactivation of microbes and is more effective in the case of thin-layer products [56, 90]. Among the spectrum of UV, UV-C was more effective in inactivating

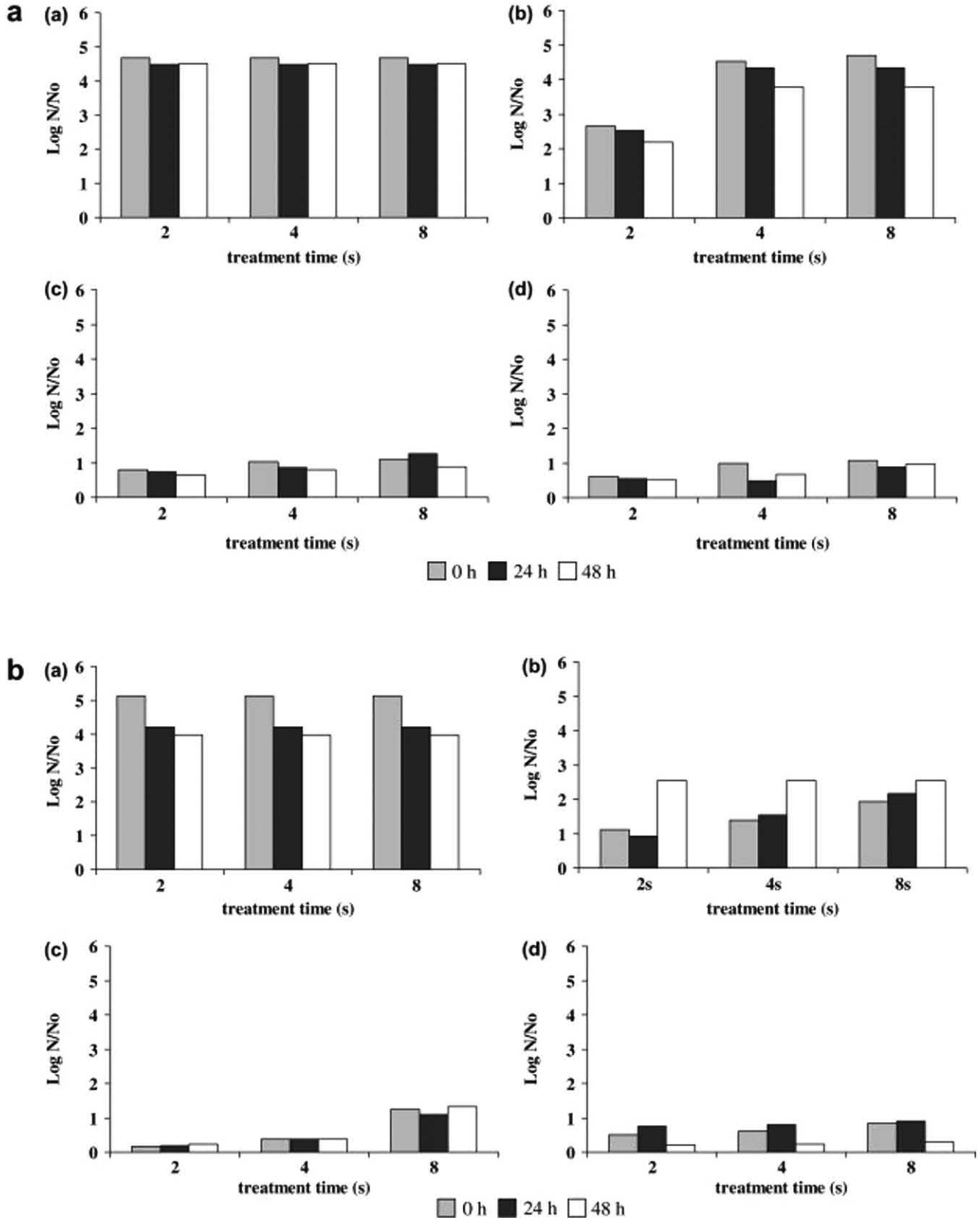

FIGURE 46.9 **a**. Effect of PL treatment duration and storage times (grey 0 h, black 24 h, white 48 h) on inactivation of *Escherichia coli* in (a) maximum recovery diluent (MRD), (b) apple juice, (c) orange juice, (d) milk. **b.** Effect of PL treatment duration and storage times (grey 0 h, black 24 h, white 48 h) on inactivation of *Listeria innocua* in (a) maximum recovery diluent (MRD), (b) apple juice, (c) orange juice, (d) milk. (From Palgan et al. [10].)

vegetative cells and spores [37, 56, 59]. It was reported that a 266 nm dose of 0.42 J/cm^2 inactivated *E. coli* in suspension by 7 log units, whereas a 355 nm dose of 16.7 J/cm^2 inactivated *E. coli* by 4 log units only [83].

During long PL treatments, the heating effect has an important role in the quality changes of the product. A lower product temperature ensures better retention of nutrients, since bioactive components present in food may be degraded during the sample heating process. Product heating and browning due to PL treatment have been reported as a major limiting factor for its application in the processing of foods [18]. There was a strong linear relationship between temperature attained and PL treatment time for apple juice, orange juice, and milk with average temperatures of 36.2, 40.6, and 40.1°C, respectively [10]. Product temperature depends on the treatment time, number of pulses, and distance from the PL source [5, 33, 88, 89]. Further, to dissipate the temperature changes due to product heating, integration of the cooling systems in the equipment can be implemented that confines the heating rate and reduces the final temperature of the product [30]. Dry PL (DPL) treatment for 60 s increased the blueberry surface temperature to 64.8°C, whereas it reached only 34°C with wet PL (WPL) [18]. Raspberries and blueberries were submerged in agitated water to remove the excess heat produced by PL, and the WPL process provided the benefits of conserving sensorial and nutrition quality by decreasing sample heating, uniform PL exposure, and physical removal of microbes due to the agitation of water [91]. For spot inoculation on blueberry, DPL and WPL treatments decreased *Salmonella* by 0.9 and 4.4 respectively (Figure 46.10), whereas for dip inoculation, *Salmonella* reduction was 0.6 and 0.8 log units respectively [92]. Thus, PL and washing process showed a synergistic effect on the inactivation of *Salmonella* spot inoculated on blueberries. For the decontamination of berries, WPL processing was reported to be one of the possible non-chemical substitutes for chlorine washing as it had greater effectiveness and an environmentally friendly process [91]. WPL treatment showed time-dependent log reduction for spot-inoculated green onions, whereas WPL treatment had practically no influence on treatment time for dip-inoculated ones [85]. *E. coli* inactivation in green onions by DPL at 5 s was more effective than 60 s WPL treatment (>4 log reduction) [85]. Therefore, light-based technology with a slight modification by the addition of cooling systems in order to reduce the thermal effect can be a promising technique to inactivate the microbes in vegetables and fruits (solid and liquid foods) by retaining the quality of foods and increasing the shelf life of the products.

PUV and PL were found to be more effective than continuous UV treatment for both aqueous suspensions and surfaces [51, 64, 93]. The turbulence has a distinctive effect on microbial inactivation, and PL efficacy enhanced considerably with increased turbulence [13, 45, 74]. Continuous flow-through PL of apple juice was more effective in inactivating microbes than batch-mode PL [32, 33, 75]. The inactivation of microbes (*E. coli*, *Salmonella*, and *S. cerevisiae*) was 1.8–2.4 log cycles in apple juice treated with continuous-flow PL (0.73 J/cm^2), whereas it was 0.4–1.7 log cycles in juice treated with batch PL (2.4 J/cm^2) [33]. In apple juice, a maximum reduction of 7.3 log CFU/ml was noticed for *E. coli* with high turbulence (3000 rpm) compared to 4.5 and 2.7 log reduction for the treatment with low turbulence (500 rpm) and static treatment respectively. For the same high turbulence treatment, inactivation counts of *E. coli* in apple cider were 5.5 log units higher than low turbulence and about 3.2 log CFU/ml greater than static treatment [13]. Inactivation of microbes also depends on the distribution of fluence on the sample surface, and location of microbes [10, 94]. The shielding of microbes from incident light by suspended matter is one of the major limiting factors in PL treatment for liquid substrates [13]. The surface topology of the product plays an important role in PL treatment, as microbes can lodge on irregular surfaces and thus inhibit the effect of PL on the target organism. Kaack and Lyager [95] noticed that PL application reduced the amount of inoculated yeast cells on carrot slices by about three to four cycles, whereas Gómez et al. [67] reported 1.6

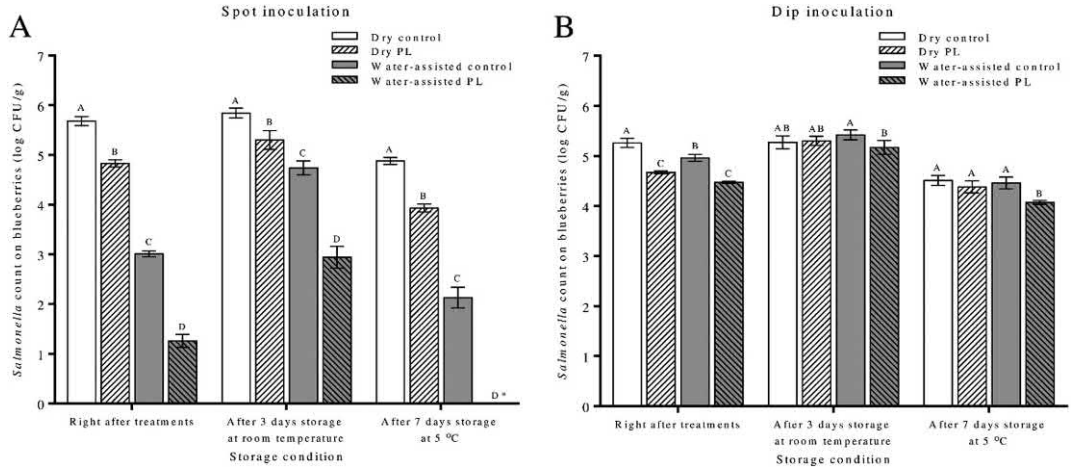

FIGURE 46.10 Counts of *Salmonella* spot-inoculated (A) or dip-inoculated (B) on blueberries after treatments and after 3 days of storage at room temperature or 7 days of storage at 5°C. Dry control blueberry samples and samples washed with tap water or treated with dry or water-assisted PL treatments were stored at room temperature for 3 days or 5°C for 7 days. (From Cao et al. [92].)

log cycles in apple discs, which can be due to the shielding of microorganisms by rough apple surface and internalization into apple tissue [96]. Likewise, the reduction in the native microflora was lesser than *L. innocua* and *E. coli* inoculated on spinach samples flashed with PL due to internalization of endogenous microorganism [73]. Raspberry and strawberry treated with PL at 5.4 J/cm^2 inactivated *E. coli* by 3.0 and 2.3 log CFU/g, *Salmonella* by 3.4 and 3.9 log CFU/g respectively, and this variation in inactivation can be attributed to the difference in the surface of the fruits [17]. The difference in efficacy of PL against *E. coli* and *Salmonella* inoculated on blueberry skin (>6.7 and 5.7 log reduction respectively) and calyx (4.3 and 4.1 log reduction respectively) was due to the rougher surface of calyx that allows more shadowing effect [18]. Xu et al. [85] also observed a difference in the inactivation efficacy of PL on *E. coli* inoculated on the stems (4.9 log CFU/g) and leaves (5.2 log CFU/g) of green onions. Similarly, Huang et al. [36] found that the reduction of *E. coli* and *Salmonella* on blueberry (5.7 and 4.2 log reduction respectively) was higher compared to that on strawberry (2.1 and 1.9 log reduction respectively) at 22.5 J/cm^2. The presence of achenes on strawberry could possibly shade bacteria from the PL beam leading to incomplete decontamination compared to blueberries with smooth skin [36]. Cauliflower, being the most irregularly surfaced vegetable, was less disinfected by PL than other fruits and vegetables, and thus the antimicrobial efficiency of PL exhibited clear dependence on surface irregularity [26]. The irregular surface of the matrix protects the hidden microorganisms from IPL so that microbial decontamination becomes limited, irrespective of the fluence applied [45, 56, 71, 84, 97]. Koh et al. [98] studied the effect of cut type on fresh-cut cantaloupe treated with PL and found that sphere samples had significantly lower microbial count compared to cuboid and triangular prism-shaped samples. The increasing microbial growth was attributed to a higher area/volume ratio which causes more wounds on the product, thus leading to higher electrolyte leakage and in turn higher microbial growth [98]. A higher degree of microbial inactivation (about 50%) was observed in sphere-shaped cantaloupe compared to that of cuboidal or triangular prism ones. This difference could be due to the decreased scattering of light around the edges of the sphere and reduced shielding influence due to lower initial microbial load [98]. At the end of the 10th day of storage, *Salmonella* counts were lower (2.3 and 2 log CFU/g) in raspberries treated with PL at 14.3 and 28.2 J/cm^2 respectively, as compared to control (5.0 log CFU/g) [65]. PL processing showed its ability to decontaminate and promote higher microbial stability during storage, thus increasing the shelf-life of the product [78, 87, 96]. PL processing could inactivate microbial growth and, thus, extend the shelf life of treated fresh-cut cantaloupe samples by 8 days [98]. Ramos-Villarroel et al. [41] concluded that PL can extend the microbial shelf life of the fresh produce, but PL in combination with appropriate packaging systems along with the application of firmness stabilizers and anti-browning agents retain the physical and chemical quality of the treated samples.

46.4.2 Meat, Marine, and Poultry Products

Microbial decontamination of animal products such as meat, poultry, and dairy products for improving safety and shelf-life has been reported, and key findings are presented in Table 46.2. The sliced ham lost less moisture when placed far away from the lamp than when located near the lamp, and there was a 6°C temperature increase with every 10 s of PUV light treatment [89]. PUV treatment also affected the tissue structure of ham, which may be due to the destruction of the network and changes in myofibrillar proteins [89]. PL-treated vacuum-packaged ham extended the shelf life by an additional 30 days compared to the vacuum-packaged-only ham [97]. Further, the inactivation of *Listeria* and *Salmonella* on chicken breast surface was found to be 0.8–0.9 log reduction at 0.8–1.1 J/cm^2 and 2–2.4 log reduction at 5.4 J/cm^2 respectively. At 5.4 J/cm^2 fluence of UV light, reduction by 2, 2, and 2.4 log CFU/ml was observed for *Listeria*, aerobic plate count, and *Salmonella* respectively on the chicken surface [25]. The reduction in microbes was similar for both chicken fillets that are unpacked and modified atmosphere packaging (MAP) packed, and treated with PUV at higher fluence (10.8 and 18.0 J/cm^2). When MAP chicken fillets were treated with lower fluence of 1.2 and 3.6 J/cm^2, they showed lower (0.9 and 0.7 log, respectively) reductions [46]. A minor elevation of temperature was noted by about 0.5–2.5 and 2.5–3.5°C when PUV at fluence of 10.8 and 18 J/cm^2, respectively, was applied to chicken samples [46]. They also found a limited dose-response effect on chicken fillet treated with PUV, due to the shading effect of uneven surface structures of the samples. The reduction of *Salmonella enterica* was lower in the case of cells inoculated in the dough before sheeting when compared to those inoculated on the surface of egg pasta after sheeting [90]. High doses of PL reduced *Salmonella* as a result of egg pasta heating or sample heating rather than the germicidal effect of the UV component of light [90]. Egg decontamination efficiency by PL could also depend on the washing process before the processing. Higher *Salmonella* decontamination was achieved in unwashed eggs than washed eggs, which could be due to damage of egg cuticle that acted as a protective shielding against PL. The washing procedures could damage the cuticle and, therefore, enable microbial cell penetration into pores, that in turn protect bacteria from being affected by PL [99]. In contrast, a 5 log reduction in *Salmonella* population was detected in both washed and unwashed eggshells flashed at 2.1 J/cm^2 PL [100]. They used a different washing procedure for eggshells (immersion in 70% ethanol) and stated that washing has no effect on the antimicrobial efficacy of PL on the surface of eggshells without any *Salmonella* penetration into egg content. *Salmonella* inoculated on eggshell was found to have photoreactivation capability, and hence it was advised to store eggs that are PL-treated away from light [99]. Meanwhile, the photoreactivation capability of *L. monocytogenes* inoculated on ham and bologna was not observed [97].

46.4.3 Milk and Milk Products

In milk, after PL treatment of 7–28 J/cm^2, *E. coli* was decreased by 0.6–1.1 log CFU/ml and for *L. innocua*, it was

0.5–0.8 log CFU/ml respectively [10]. A high fluence of 26.2 J/cm^2 resulted in 3.2 log reduction in the total microbial count, and concomitantly, milk temperature was increased to 55°C which indicated a combined effect of photochemical and photothermal damage of natural microflora by PL in raw milk [101]. The milk with different total solids (9.8, 25, and 45 %) treated with PL of 2.1 J/cm^2 reduced *E. coli* by 0.21, 0.13, and 0.03 log units, respectively [74]. No significant difference in inactivation of *E. coli* was observed when milk with different fat levels (skim, 2% fat, and whole milk) was treated with PL. The inactivation levels of *E. coli* decreased by 2.0, 0.6, and 0.4 log CFU for 9.8, 25, and 45% total solids, respectively, as the total solids in reconstituted milk increased. PL treatment efficiency was hindered by the presence of milk fat due to the scattering of light by fat globules [74]. Increasing levels of oil and protein reduced the killing efficiency of IPL, since proteins have high absorption at about 280 nm and lipids absorb a higher wavelength of the UV-B region, thus decreasing the effective radiation dose on microbes. Since starch did not show any negative impact on the decontamination of microbes, foods such as fruits and vegetables, which are high in carbohydrates, but poor in fats and proteins, seem to be more appropriate for IPL processing [102]. The use of turbulence enhanced the inactivation of *E. coli* in reconstituted milk by PL treatment [74]. Thus, turbulence with thin layer flow can significantly improve the effectiveness of PL treatment, presumably by maximizing exposure of microbial cells to the incident light, and could disintegrate the clusters of microbial cells that lead to the increase of microbial inactivation. A 3 log reduction of *L. innocua* was found on Gouda cheese samples flashed with just 0.9 J/cm^2, whereas a 1 log reduction was found for Manchego cheese slices [103]. The difference in decontamination magnitude between types of cheese was noticed, and this could be described by their different topography (i.e. the porous nature in Manchego vs smoothness of Gouda-type cheeses) [103]. A *L. innocua* reduction of 3 log CFU/slice was observed in cheddar and processed cheese slices treated with 9 J/cm^2 [104].

46.4.4 Other Foods

The inactivation of *L. monocytogenes* in infant meals was effective (3 log reduction at 4800 μs), but lower than infant beverages (5 log reduction at 3500 μs) which is due to the product characteristics [23]. Greater PL treatment time (75 s) significantly impaired seed viability by 54 ± 17 and 46 ± 9% in dry and wet barley grains respectively [76]. These results indicated that the decontamination efficacy of IPL is also closely interrelated to the moisture content of the product [23]. A significant reduction of *B. subtilis* spores (4.2) was obtained with a fluence of 1.5 J/cm^2 PL in 65°Brix sucrose syrup [12]. This study also indicated that the increase in depth of sugar syrup from 3 to 10 mm did not significantly affect the *B. subtilis* spore inactivation. Hwang et al. [71] reported a 0.99 log reduction of inoculated microorganism (80% bacteria + 20% molds and yeast) on sesame seeds when IPL treatment was applied, whereas a 7 log reduction of *P. aeruginosa* was noticed in inoculated mineral water [28]. This large change in reduction is mainly due to the matrix type: all sides of sesame seeds could not be exposed to IPL because of the shadowing effect. The addition of a mixing step during IPL treatment of sesame seeds reduced the microbes by 1.02 log units at 39.85 J/cm^2 compared to 0.86 log reductions without a mixing step [71]. Likewise, Moreaua et al. [105] showed that the reduced efficiency of PL in the case of peppercorn decontamination compared to that of inactivation of *B. subtilis* on glass marbles could be attributed to the non-uniform surface of the spice. The rough rice treated with PL (84.4 J/cm^2) exhibited a reduction of aflatoxin B_1 (AFB_1) from 132 to 32 ppb (75% reduction) and aflatoxin B_2 (AFB_2) from 45 to 27 ppb (39% reduction) [106]. However, in rice bran irradiated with PL of 16.1 J/cm^2, the degradation of AFB_1 and AFB_2 reduced by 90.3 and 86.7%, respectively, which was higher than rough rice due to the thinner structure of rice bran. AFB_1 was found to be more susceptible to PL degradation than AFB_2 probably due to the presence of C_8–C_9 double bond in the furan ring of AFB_1, which is liable and vulnerable to photodegradation [106]. Meanwhile, it is noteworthy that UV wavelength spectrum and PL intensity are major factors that affect the degradation of aflatoxins and the rate of degradation was greater for PL treatment as compared to conventional UV treatments [106]. A significant inactivation of *S. cerevisiae*, *A. niger*, *B. subtilis*, *G. stearothermophilus*, and *A. acidoterrestris* spores (5.4, 1.3, <4, and 3 log reduction respectively) was obtained with a fluence of 1.6–1.9 J/cm^2 PL in 65°Brix sucrose syrup [12]. Ground caraway and ground black/red pepper flashed with PL of 10 J/m^2 reduced *B. subtilis* by 0.86–1.0 log units [45].

46.5 Effect of Pulsed Light on Physicochemical Characteristics of Food Products

46.5.1 Physical Properties

The external appearance of grapes was not affected by the application of PL before crushing, whereas after crushing, the pulp was slightly darker than control, due to the diffusion of anthocyanin into the berry [107]. Immediately after the PL treatment, no significant change in color was noticed on fresh-cut avocado treated at 6 J/cm^2, but there were signs of browning in samples treated at 12 J/cm^2 [41]. The net color changes of fresh-cut avocados treated with PL were higher than the control ones and were closely related to the fluence applied; thus the intense application of PL has a more noticeable impact on the color of the samples [41]. Fresh-cut avocados treated with PL did not seem to avoid enzymatic browning, but maintained better hue angle values over time compared to untreated avocado pieces. Meanwhile, chlorophyll content after PL application (6 J/cm^2) on fresh-cut avocados was 1.3-fold higher than in untreated samples [108]. The lightness of the PL-treated fresh-cut avocados decreased throughout the storage period (15 days) and was lower than that of untreated samples [41, 58]. However, variation in color by a shift in a*

values of the endive salad, and fresh-cut avocados treated with PL was more distinct as the fluence applied intensified [56], leading to browning during storage and firmness was also significantly affected [41]. In contrast, PL had no adverse effect on the color of mung bean sprouts, and rather had a positive effect on overall appearance which may be because of lower microbial population [56]. Meanwhile, UV spectra affected the color and texture of fresh-cut mushroom and cut avocados and, therefore, treating fresh produce with quality-stabilizing agents (anti-browning and texture stabilizers) before PL flashing can be recommended for extending the shelf life of a product [40, 58]. Weight loss and browning of apple slices were observed with higher PL treatment (17.5 J/cm^2) due to rupture of membrane and loss of turgidity [87]. Fresh-cut apples treated with PL exhibited a slight decrease in L* values, were susceptible to surface browning, and had lower firmness values when compared to untreated apple samples [96]. Apple slices exposed to PL turned darker and less green than the control, and this influence was more evident as PL dose and storage time increased, probably initiated by browning reactions enhanced by temperature increase during irradiation [67]. PL induced degradation of biopolymers in cell wall, affecting the pectin present in the cell wall, and cells appeared collapsed with ruptured membranes, thus causing a rupture, and folding of cell walls. This membrane damage would increase enzymatic browning reactions due to the loss of functional cell compartmentalization and tissue damage [67]. PL treatment did not affect pH, °Brix, and non-enzymatic browning index, whereas it slightly affected the color of apple juice [32]. Fresh-cut mangoes flashed with PL maintained the color and firmness during 7 days of storage as the control [109]. Green onions treated at 30 s and 60 s DPL were observed to have an alteration in quality with softer and shrunken tissue, color, and smell [85]. Raspberries treated with PL showed a decrease in brightness and did not substantially change the redness of the fruit during storage, and instability of firmness of the fruit was also observed [65]. The DPL treatment degraded the blueberry wax and resulted in a burnt appearance with severe discoloration of blueberries. Color discoloration was coupled with sample heating when blueberries were treated with DPL for 60 s when compared to WPL [18]. The L* value of blueberries treated with WPL, decreased during the storage period (3 and 7 days at room temperature and 5°C, respectively) by losing the surface glossiness and formation of white bloom on the surface [92]. However, color was not negatively affected by PL treatment for fruits and vegetables [26, 87, 98].

PL treatment at 2.1 J/cm^2 decreased the redness and increased the lightness of ham, but in bologna samples, PL at 4.2 J/cm^2 decreased the lightness and increased the yellowness [97]. No significant change in color and total score values was observed in beef samples treated with PL [110]. PL considerably affected the yellowness and redness of the beef *carpaccio* when treated with ≥8.4 J/cm^2, but lightness was not altered between PL-treated and untreated samples. During shelf-life studies, beef and tuna *carpaccio* showed significant, remarkable differences in color and odor when treated with PL at 4.2 J/cm^2 and above [44], whereas Tomašević [110] found the difference in odor of beef samples at 3.4 J/cm^2. When *salchichon* was flashed with 11.9 J/cm^2 fluence, there was no significant difference in color, odor, and flavor, but for dry-cured loin, all these quality parameters changed [47]. Color parameters were not dramatically modified by PL treatment in these ready-to-eat (RTE) dry-cured products, which may be attributed to the greater stability of the cured pigments in comparison to those of fresh meat [47]. The sliced ham flashed with PUV showed a slightly darker color, and decreased both a* and b* values compared to control at the end of 14 days of storage [89]. The vacuum packaging of chicken frankfurters did not have any effect on the log reduction of *L. monocytogenes* (maximum of 1.5 log CFU/ cm^2) with UV treatment (60 s at 8 cm); however, color and quality changes in products were observed [111]. A higher dose of PL (>1.75 J/cm^2) treatment had a slight effect on the appearance and color and enhanced the formation of non-enzymatic browning products and in turn, increased the oxidative stability of egg pasta [90]. Cheddar cheese flashed with PL maintained a stable lightness value L* and lipid peroxide value over 30 days of refrigerated storage [112].

External damage was not observed on strawberries treated with PL (250 s), and the firmness of the treated fruit was similar to that of control berries [55]. Strawberries flashed with PL did not display any pronounced softening when compared to untreated samples even after 8 days of storage at 6°C, and cell wall strengthening of fruit was induced by PL stress [81]. Meanwhile, TEM studies also showed that the internal tangential walls strengthened as did the integrity of the walls of hypodermis cells of fruit by PL stress, whereas the loss of cell assembly was observed in untreated stored strawberry fruits [81]. DPL- and WPL-treated blueberries exhibited shrinkage at the stem of the fruit, showing probable moisture loss during the PL treatment [92]. WPL-treated blueberry samples exhibited significantly lower firmness values than the DPL and water-assisted control samples [92]. Contrastingly, PL at fluence of 8 J/cm^2 drastically affected the texture of fresh-cut tomatoes (55.7% loss). The firmness of the PL (4 J/cm^2) treated fresh-cut tomatoes did not show any significant modifications immediately after the treatment, whereas throughout the storage period (20 days) the firmness values decreased at a higher rate of about 72% [82]. Mango slices treated with PL decreased in firmness of the sample after processing more than untreated ones [94]. Firmness values of fresh-cut avocado treated with IPL decreased intensely compared to control samples immediately after processing, displaying that the UV portion of IPL affected the softness of the fruit [58]. The use of PL (14 and 28 J/cm^2) dramatically affected the textural quality of fresh-cut mushrooms by increasing the toughness of the product, dehydration, and major textural modification [113]. Thus, PL treatment affected the textural properties and firmness of fruit and vegetables [26, 65, 87, 96]. Firmness retention was also observed in PL-treated fresh-cut cantaloupe compared to untreated samples during storage, which may be due to the thickness of the sample (~3 cm) as the effect of PL is restricted to the surface of the product [98, 114]. But contrarily, the adverse effect of single PL treatment at 11.7 J/cm^2

on tissue structure of fresh-cut cantaloupes under chilled storage was minimized by applying repetitive PL (RPL) treatment at 0.9 J/cm^2 every 48-h interval leading to increased microbiological quality, and retention of firmness and ascorbic acid content [114]. Further, firmness was higher for fresh-cut cantaloupes treated with RPL compared to the untreated fresh-cut cantaloupes throughout storage, which may be due to lower CO_2 concentration in treated samples, which could otherwise de-compartmentalize the enzyme and its substrates and then act on cell walls of fruit tissue leading to rapid deterioration [114]. It was also noticed that the PUV-treated sliced ham decreased hardness over the storage time (14 days) which may be due to destruction of the network of tissues that resulted in softening [89].

The results of the sensory studies conducted by Palgan et al. [10] on reconstituted apple juice exposed to PL at 28 J/cm^2 fluence showed that there was no significant difference in terms of color, sweetness, odor, or acidity of apple juice, but the lowest score was observed for flavor compared to either control or samples PL treated for a shorter time. Off-odor in PL-treated samples remained over the 14 days' storage period due to photophysical changes that occurred on fresh-cut apples [96]. Further, slight changes in flavor were noted by Ignat et al. [87] in apple slices exposed to a fluence of 17.5 kJ/cm^2, which was similar to those detected during the 7-day storage of untreated ones. The effect of PL on grape processing was said to decrease the herbaceous and oxidative smell, so it may be that PL is able to degrade the compounds responsible for green notes and oxidative flavors in wines [107]. UV light is known to induce a series of adverse effects in food products due to the generation of free radicals through diverse photochemical reactions, which can damage vitamins and antioxidants, while also inducing lipid oxidation and color changes [115]. Hierro et al. [97] mentioned that the sensory parameters of ham slices were not altered, whereas for bologna, a difference in odor and flavor profile was observed at fluences higher than 4.2 J/cm^2. In contrast, PL fluences of 8.4 J/cm^2 and ≥4.2 J/cm^2 affected the raw attributes of beef, and tuna *carpaccio* respectively, by inducing the development of sulfur notes [44]. Sensory properties on the chicken breast treated with PL showed no visual color changes, no raw meat flavor, and taste changes, even though there was an increase in the surface temperature up to 38°C after application of 5.1 J/cm^2 UV dose [57]. Most remarkably, unpackaged chicken fillets treated with high fluence PUV (10.8 J/cm^2) showed the highest intensity of sunburnt odor and flavor, which was due to increases in compounds such as 2-pentanone and 1-pentanol [46]. At higher fluence (>6 J/cm^2), thermal effects were induced (54°C), and in turn changes in organoleptic properties of chicken breast meat were perceived [25]. Lasagabaster et al. [100] observed that PL (10.5 J/cm^2) had no effect on the rheological properties of egg and the slight burnt odor was perceived as a sensory parameter, which was not significant. Even PUV application on eggshells had no effect on egg quality in terms of albumen height, egg deformation, eggshell strength, color (L*, a*, and b* values), and the presence of cuticle [116]. Orange pigments were decreased in egg pasta immediately after PL with intense light dose of 26.2 J/cm^2 [90]. In Gouda and Manchego cheeses, there was a significant difference in the odor and flavor in slices treated with 4.2 and 8.4 J/cm^2, indicating the presence of sulfur notes [103]. In cheddar cheese, PL did not affect the color and lipid peroxidation during refrigerated conditions. However, a dose of 9.22 J/cm^2 had an adverse effect on organoleptic properties of cheese [112]. Sensory analysis of cheddar cheese treated with PL showed hedonic scores for liking, flavor, and appearance nearer to untreated control samples; thus Proulx et al. [112] mentioned that PL can be used for commercial application to minimize the undesirable sensory effects on cheese.

46.5.2 Chemical Properties

The enhancement of extraction in grape flashed by PL was observed by the increase in levels of methanol, which is a consequence of the photothermal effect of PL on the grape skin structure [107]. The total soluble solids (TSS) of mango slices treated with PL (8 J/cm^2) did not decrease as compared to untreated slices [94]. Koh et al. [98, 114] even found that there was no effect on TSS of fresh-cut cantaloupe at 4 ± 1°C due to a decreased respiration rate in chilled storage. The increase in acidity was more pronounced in untreated fresh-cut cantaloupes and avocados compared to PL-treated ones, throughout the storage [41, 116]. Maftei et al. [84] found no change in color, soluble substances, and pH in the apple juice treated with PL (53.3 J/g). PL treatment did not have any effect on the antioxidant activity of fruits and vegetables [26, 96]. Even though the anthocyanin content was higher after crushing, the levels were similar to those of controls at the end of the fermentation [107]. Total ascorbic acid content (AAC) and polyphenolic content were similar in both untreated and PL-treated fresh-cut mango samples during 7 days of storage [109]. AAC was conserved throughout the storage period in cut cantaloupe treated at low fluences of 2.7 and 7.8 J/cm^2 [98]. A slight increase in AAC after RPL treatment in cut cantaloupes was noticed and maintained throughout the storage, which could be due to abiotic stress exerted by PL irradiation [114]. IPL applied to spinach and RPL on fresh-cut cantaloupes led to an increase in total phenolics concentration and antioxidant capacity, which may be due to free radical formation by PL stress response that increases the antioxidant production [73, 114]. However, during refrigerated storage, the polyphenol content and antioxidant capacity decreased drastically, and PL-treated spinach was similar to control at the end of storage [73]. The PL treatment of fresh-cut mangoes increased polyphenol oxidase (PPO) activity after 3 days and maintained phenylalanine ammonia lyase activity [109]. IPL treatment did not affect AAC in fruits and had a negligible effect on total phenolic content (TPC) in fresh fruits and vegetables [26, 65, 98, 109, 113]. Similarly, even the mushroom samples treated with 28 J/cm^2 reduced in vitamin-C content and antioxidant capacity compared to 4.8 and 12 J/cm^2 flashing [113]. The TPC of WPL-treated blueberries and water-assisted control samples were significantly higher than the dry control samples immediately after the treatment. However,

blueberries treated with PL showed a lower level of anthocyanin content than dry control samples after 3 days' storage at room temperature [92]. The total anthocyanin content (TAC) of raspberry was not influenced by PL at 5 s and 15 s; surprisingly PL-treated berries showed higher TAC compared to the control at the end of the 10-day storage. However, PL at 30 s increased the TAC by 10.1 mg cyanidin-3-glucoside equivalents/100 g fruit, when compared to 5 s treatment which could be due to stimulation of color and anthocyanin accumulation by PL [65]. Higher concentrations of β-carotene were observed after PL processing of carrot slices (fluences of 2.26 and 4.52 J/cm^2) as compared with control samples, and this behavior was also partly associated with the intense color noticed in the cortex tissue of samples [117]. Further, the carotenoid content of PL treated fresh-cut mangoes was four times higher than the control mangoes on the 7th day of storage [109].

The respiration rate of endive salad, spinach, and fresh-cut cantaloupe was increased by production of CO_2 and O_2 consumption at a higher rate when PL was applied [56, 73, 98]. Similarly, partial pressures of O_2 decreased (by ~5 kPa) and that of CO_2 increased (by ~0.7 kPa) inside the packages of tomato slices on PL processing [78]. These changes could be associated with physiological stress or even physiological damage caused by the IPL treatment, which could affect the metabolic activity of the vegetable/fruit tissue. The changes in headspace gas of the food products were influenced by the fluence applied, storage period, and spectral distribution of IPL treatment [58]. The fresh-cut avocado treated with 305–1100 nm exhibited higher CO_2 concentration than those treated at the 400–1100 nm range, indicating that the UV portion of light led to the generation of anoxic conditions in the headspace of the avocados [58]. The UV part of PL was primarily responsible for a hastened quality loss due to greater respiration rate [56]. IPL treatment increased the respiration rate and gas concentration of lettuce and fresh-cut mushroom at the end of the storage. The O_2 level was less than 2%, indicating anaerobic respiration which affected the sensorial properties of the product [40, 102]. During storage, the respiration rate of IPL-treated fresh-cut avocados increased due to an increasing wounding response and thus caused an undesirable anaerobic condition triggering anaerobic metabolism in the fruit [41]. This anaerobic metabolism leads to a fermentation process, increasing the ethanol production, and inhibits ethylene production in treated fruits [41]. In contrast, PL application on cantaloupe and mung bean sprouts did not result in any significant effect on CO_2 production [56, 114].

One of the disadvantages of continuous UV light is the induction of oxidation processes in meat, which changes its sensorial properties [25, 57, 89]. Ham and bologna treated with PL (above 2.1 J/cm^2) were found to reflect very low lipid oxidation [97], whereas, PUV light treatment induced an oxidation process in sliced ham thus making slices rancid during the storage time (14 days), which may be due to the oxidation process stimulated by the heat generated during the process [89]. PL treatment of egg pasta did not affect peroxide value; however the treatment helped to decrease the formation rate during the storage period [90]. Chicken breast meat flashed with PL showed slightly higher lipid peroxidation (0.16 mg MDA/kg meat) than the control samples [25, 116]. Lipid peroxidation in PL-treated vacuum-packed fermented sausage slices and samples packed in modified atmosphere were not significantly different from the control [40]. These findings showed that light-based processing of food products with proper packaging has an application in food industry to increase the shelf life and to maintain the organoleptic properties of food during storage.

46.6 COMBINED TREATMENTS

The limitations of PL processing are uneven exposure, shadowing effect, browning, and product heating. As a result, many hurdle technology strategies have been developed to address and challenge the limits of PL processing [118]. Combinations of processes have been attempted to increase the microbial inactivation rate and overall process efficiency of PL-based processes [55].

46.6.1 Ultrasound and Pulsed Light

A combination of US and PL resulted in 5.8 and 6.4 log reductions in *S. cerevisiae* KE 162 population, in naturally squeezed and commercial apple juice, respectively [63]. When PL (12 J/cm^2) was applied prior to US treatment, a 6 log reduction in *S. cerevisiae* population was observed in the above two juices. Another study reported that a combinational process provides a synergistic effect on microbial inactivation [119]. US in combination with PL (continuous mode) treatment on commercial apple juice exhibited a synergistic effect that was higher than any of the individual treatments can achieve, i.e. 6.3, 5.9, and 3.7 log reductions for *S. enteritidis*, *E. coli*, and *S. cerevisiae*, respectively [33]. The combination of US and PL (i.e. continuous mode) exhibited the lowest luminosity, decrease in a*, and increase in b* values with respect to control apple juice immediately after the treatment. Additionally, color retention was observed after the combination treatment by preventing apple juice from turning darker and brownish, and also delaying the mold and yeast recovery during the 10 days' storage period [33]. The apple juice treated with US+PL (continuous mode) showed adequate acceptability and fresh natural apple flavor as the main attribute that was highlighted by consumers during sensory evaluation [33].

Muñoz et al. [120] reported that the combination of high-intensity light pulses (HILP) and thermosonication (TS) regardless of the sequence applied had an additive effect on inactivation of *E. coli* in orange juices when compared to either of the processes as a stand-alone. Moreover, TS technology used as a first hurdle, followed by HILP, achieved a significant reduction in *E. coli* population in orange juice when compared to untreated juice or TS as an individual treatment [120]. The combination of PL and TS on apple juice led to significant inactivation of *E. coli* by 4.9 and 5.9 log units when TS was applied at low (14 ml/min, 40°C) and high (8 ml/min, 53°C) settings, respectively. This additive effect on

microbial inactivation could be due to the impact of treatment on different targets, that is, DNA for PL and cell membrane for TS [32]. The combination of PL+TS induced the maximum decrease in L* and b* values and minimum decrease in a* values compared to the reverse treatment, TS+PL [32]. Muñoz et al. [120] reported that there was no sign of sublethal injury to cells with HILP, both at low (4.03 J/cm^2) and high (5.1 J/cm^2) intensities and TS applied individually or in combination. Sublethal damage was detected by Muñoz et al. [32] when PL (low) was applied as the first hurdle, as compared to PL (high) treatment.

46.6.2 Pulsed Electric Field and Pulsed Light

The sequence of HILP/pulsed electric field (PEF) resulted in a slight lowering of *E. coli* K12 cells in apple juice compared to PEF/HILP, but a combination of HILP and PEF suggested a synergistic effect on the inactivation of *E. coli*. This combination had no effect on quality parameters like pH, Brix, browning index, and total phenolics, except for slight color changes [31]. The combination of HILP and PEF treatment had a significant effect on sensory attributes such as flavor and odor of the non-thermally treated apple juice, as compared to thermally pasteurized control [31]. Light-based technology (UV/HILP) combined with PEF had no effect on color, flavor, non-enzymatic browning, TPC, and TAC of the apple and cranberry juice blend and received a similar sensory score to pasteurized samples [121]. The combination of UV/HILP with manothermosonication adversely affected overall acceptability of the apple–cranberry juice blend by increased darkening, lowered flavor/odor values, and changing the overall color of the product [122].

46.6.3 Coatings and Pulsed Light

The combined application of edible coating (gellan-gum based [0.5% w/v] coating enriched with apple fiber) and PL (12 J/cm^2) treatment on fresh-cut apples retarded the microbiological deterioration, and reduced softening and browning during 14 days of storage at 4°C [123]. The combination of PL and gellan gum-based prebiotic edible coating on fresh-cut apples showed the antagonistic effect by hindering the microbial inactivation caused by PL. The uncoated fresh-cut apples treated with PL exhibited lower microbial count after 14 days of storage. Fresh-cut apples treated with PL and edible coating incorporated with apple fiber reduced the signs of oxidation by losing 68% of antioxidant value, while the sample only treated with PL showed a decrease of 83% which was similar to untreated samples [123]. Untreated and PL-treated apple slices showed higher L values than the samples with gellan gum coatings, whereas firmness was better maintained in gellan gum-coated apple slices. Overall, the combination of PL and gellan gum coating on apple slices lowered the sensory acceptability in terms of aroma and flavor during 14 days of storage [123]. In a similar study, pectin-coated fresh-cut apples that were exposed to PL were found to have the highest reduction in yeast and mold, mesophilic and psychrophilic aerobic counts during storage [96]. Pectin-coating of apple cubes (incorporated with apple fiber) was found to inhibit browning and lead to slightly higher antioxidant activity than in the untreated ones during storage [96]. Fresh-cut apple wedges dipped in quality-stabilizing solution (N-acetylcysteine dip) when treated with PL (16 J/cm^2) showed reduced mesophilic and psychrophilic microbial counts by 1.55 log units and molds and yeast loads by 2.3 log units [124]. To minimize the browning on PL-irradiated apple surface, an ascorbic acid (AC)/calcium chloride solution was used as an anti-browning dip prior to PL treatment [125]. The application of AC at 1% on sliced mushroom and apple cubes before flashing with PL significantly reduced browning during storage [113, 123]. The dipping of fresh-cut apples in AC/calcium chloride solution before pectin coating, followed by PL treatment was more efficient in minimizing browning, retaining antioxidant activity, and even did not have any effect on microbial loads and sensory acceptability of apple cubes. Firmness was also maintained in fresh-cut apples when treated with AC/calcium chloride solution as it actually helps in crosslinking the polymer matrix and delays softening of apple surfaces [96]. It is also reported that PL treatment of fresh-cut apple tissues promotes the development of browning, especially after the application of high-energy dosages. The firmness of fresh-cut apples treated with PL of 4 J/cm^2 doubled during 15 days of storage compared to those flashed with 12 J/cm^2 [124]. Llano et al. [124] also observed that fresh-cut apples exposed to the highest fluence (8–16 J/cm^2) retained better antioxidant capacity, and total phenolic and flavonoid content throughout the 16 days of the storage period compared to the samples only dipped in quality-stabilizing solution.

Fresh-cut mushrooms dipped in AC solution exhibited 1.29, 1.03, and 0.72 log reductions in aerobic mesophilic, psychrophilic, and yeast and mold counts, respectively [113]. Application of non-thermal PL treatment against *L. innocua* inoculated on modified chitosan (MC) containing a nanoemulsion of mandarin essential oil-coated green beans (1.2×10^5 J/m^2 per bean side) resulted in a 2 log reduction. Further, PL did not show any synergistic antimicrobial effect against *L. innocua* during storage, and there was a slight detrimental impact on color with the formation of browning spots [126]. The combination of MC coating and PL treatment reduced *E. coli* population on fresh-cut cucumber slices by 1.4–3.8 log CFU/g at 4–12 J/cm^2 [127]. Meanwhile, a combination of PL with MC plus carvacrol essential oil at 3% coating was also more effective in the inactivation of *E. coli* on cucumber slices by 1.9–5.9 log CFU/g respectively for the fluence of 4–12 J/cm^2 [127]. The treatments combining PL (12 J/cm^2) and malic acid (MA) of 2% v/v resulted in significantly greater inhibition of *L. innocua* and *E. coli* populations than either PL or MA alone, by achieving a more than 5 log reduction for fresh produces such as fresh-cut avocado, watermelon, and mushroom throughout the storage period [48]. Even TEM observations confirmed that damage, especially to *E. coli* cells, was caused by a combination of treatments due to agglutination of cytoplasmic content and disruption of cell membrane causing cell death [48]. The application of PL-alginate coating

(ALC)-MA, and ALC-MA-PL treatments on fresh-cut mango led to 3.92 and 3.03 log reductions of *L. innocua* counts, respectively, whereas a combination of MA and PL showed an additive effect on *Listeria* reduction by 4.49 log units compared to PL alone (2.5 log CFU/g) [94]. This difference can be explained by the antagonistic action attributed by ALC, that limits the effect of MA and PL. Meanwhile, the highest inactivation of molds, yeast, and psychrophilic bacteria was obtained with PL-ALC-MA treatment which showed the best microbial quality of mango slices during storage, and ALC helped to maintain the integrity of fruit by reducing the presence of exudates. Therefore, PL should be applied before ALC and MA treatment to overcome the interference caused by ALC [94]. The treatment of mango slices with PL individually was not found to cause any change in pH, but ALC-treated samples increased in pH values and decreased in L values of color in comparison with control samples [94].

The application of anti-browning dipping treatment in combination with IPL would increase the shelf life of minimally processed vegetables and fruits [102]. The coating application after PL treatment would have more influence on the surface texture than if applied alone, since PL may improve the permeabilization of the cell wall of the fruit prior to coating application [94]. The application of PL-ALC-MA and ALC-MA-PL treatments on fresh-cut mango resulted in the highest radical scavenging activity (RSA) of about 42.2 ± 0.4% RSA at the end of 14 days of storage [128]. The PL and MA treatments on fresh-cut mango slices did not show any significant effect on phenolic content compared to the untreated ones; contrarily, mango slices treated with ALC and ALC-MA-PL showed higher content of phenolic compounds immediately after processing as well as at the end of the 14-day storage period [128]. Even, the color of the mango slices was retained after the application of PL, ALC, and MA treatments in combination, with a fresh-like color throughout the 14 days by avoiding browning due to the higher phenolic content achieved [128].

46.6.4 Other Treatments

Research findings have indicated that studies should be aimed at evaluating strategies based on the combination of PL treatments with other processing technologies, e.g. addition of natural preservatives or mild heat treatment, in order to successfully tackle safety issues for clarified juices treated with PL technology. The combined effect of PL+nisin treatment in RTE sausages resulted in a significantly higher reduction (4.03 log CFU) of *L. innocua* compared with individual treatment (PL alone: 1.37 and nisin dip alone: 2.35 log CFU). This combination of PL and nisin was said to cause damage to DNA and membrane pore formation, respectively, thus preventing the recovery of sublethally injured cells [14]. The combination of WPL and 1% hydrogen peroxide (H_2O_2) was found to be the most efficient treatment for inactivating *Salmonella* on raspberries and blueberries by >5.6 log CFU/g [91]. Raspberries treated with WPL (60 s) in combination with 100 ppm sodium dodecyl sulfate (SDS) or 1% H_2O_2 (60 s) inactivated *E. coli* by 5.1 and 5.3 log CFU/g, respectively, whereas 100 ppm SDS or 1% H_2O_2 individually reduced *E. coli* by 2.5 log CFU/g [129]. For strawberries and raspberries, WPL and WPL-H_2O_2 achieved higher log reductions of *Salmonella* (2.4–4.9 log units) than chlorine water washing (2.1 log units), proving the antimicrobial efficacy of WPL [129]. A significant synergistic effect on the inactivation of *B. subtilis* spores (>5 log reduction) was achieved with simultaneous treatment with PUV and H_2O_2 photosensitizer solution on both surfaces and aqueous solutions compared to sequential treatment [51]. Dip-inoculated green onions were treated with PL (60 s), surfactant (SDS) sanitizer combination washing (10 ppm chlorine + 1000 ppm SDS and 300 ppm H_2O_2 + 1000 ppm SDS), as well as a PL–surfactant–sanitizer combination (10 ppm chlorine + 1000 ppm SDS + 60 s PL and 300 ppm H_2O_2 + 1000 ppm SDS + 60 s PL). The combination of chlorine washing and WPL treatment had an additive effect on the inactivation of *E. coli* on green onions by about 2.4 log reductions when compared to chlorine washing or PL treatment alone [85]. H_2O_2 was slightly more capable of inactivating *E. coli* on green onions (0.7 to 2.6 log CFU/g) than citric acid and thymol in combination with 60 s PL treatment. The PL–surfactant–sanitizer combination did not show any synergistic effect on the inactivation of *E. coli* on green onions when compared to the PL–surfactant combination. However, PL in combination with SDS was more effective compared to PL with chlorine washing, thus recommending the application of a surfactant–PL combination treatment in the decontamination of fresh produce [85]. The combination of PL and heat (40 and 45°C) had a significant influence on *B. cinerea* fungal development, but the treatment sequence and temperature level didn't have any effect on the fungal growth [55]. However, a UV-C and PL combination did not show any significant effect on *B. cinerea* fungal growth. In apple juice, a combination of cold storage and PL maintained the log reduction of *S. cerevisiae* over 9 days and retarded the yeast recovery until the third day of storage [54]. The combination of post-cold storage (5°C) with PL had a significant synergistic effect on the decontamination of *L. innocua* and *S. enteritidis* in apple juice, achieving a 5–8 log reduction, and thus, PL coupled with low-temperature storage can provide microbial safety in fruit juices [54]. PL is more effective in decreasing microbial loads on a fresh-cut salad compared to similar treatments with electrolyzed water (40 ppm free chlorine) or chlorine dioxide (15 ppm). PL may, therefore, be an appropriate measure to reduce the required amount of fresh water in fresh produce processing and to keep microbial loads in the wash water at a low level [43, 130]. Thus, PL in combination with other hurdle technologies can be a novel tool for producing microbially safe foods without compromising the nutritional and sensorial quality of foods.

46.7 CONCLUSION

PL technology is a promising non-thermal technique for the decontamination of foods. The mechanism of action of PL for microbial inactivation is reported to have a combined effect of photophysical and photochemical properties (i.e. damage of the cell membrane, cytoplasm, and DNA). The efficacy of

microbial inactivation depends on several parameters, such as light pulse/energy dose, thickness/morphology of food, type of microorganism, and properties of food. The limitations of PL processing are (i) poor penetration of light, and (ii) non-uniform (i.e. inhomogeneous) decontamination. These limitations can be overcome to some extent by employing multidirectional IPL or even sample rotation. Most of the researchers have reported batch-type PL processing units for surface sterilization of products, such as meat, fruits, and vegetables, while a continuous PL system was applied to decontaminate liquid foods. Vegetative bacteria are sensitive to PL as compared to spores, viruses, and fungi. PL technology when combined with other nonthermal technologies like PEF and US could enhance microbial reduction and increase nutrient retention.

ABBREVIATIONS

PL	Pulsed light
FDA	Food and Drug Administration
UV	Ultraviolet
VIS	Visible
IR	Infrared
PUV	Pulsed ultraviolet
IPL	Intense pulsed light
MNV	Murine norovirus
DNA	Deoxyribonucleic acid
TEM	Transmission electron microscopy
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
CFU	Colony forming units
DPL	Dry pulsed light
WPL	Wet pulsed light
MAP	Modified atmosphere packaging
AFB	Aflatoxin B
RTE	Ready-to-eat
RPL	Repetitive pulsed light
TSS	Total soluble solids
AAC	Ascorbic acid content
PPO	Polyphenol oxidase
TPC	Total phenolic content
TAC	Total anthocyanin content
US	Ultrasound
PEF	Pulsed electric field
HILP	High intensity light pulses
TS	Thermosonication
AC	Ascorbic acid
MA	Malic acid
MC	Modified chitosan
ALC	Alginate coating
H_2O_2	Hydrogen peroxide
SDS	Sodium dodecyl sulphate

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47 Irradiation Preservation of Foods

Mohammad Shafiur Rahman

CONTENTS

47.1 FOOD IRRADIATION PROCESS

The irradiation process involves exposing the food, either prepackaged or in bulk, to a predetermined level of ionization radiation. In this process, it is important to know sources of ionization radiation, how energy is quantified, and its scope with advantages and limitations.

47.1.1 Action of Ionization Irradiation

Ionization radiation interacts with an irradiated material and ionizes molecules by creating positive and negative ions by transferring energy in the electrons [1]. The radiation effects on biological materials are direct and indirect. In direct action, the chemical events occur as a result of energy deposition by the radiation in the target molecule, and the indirect effects occur as a consequence of reactive diffusible free radicals formed from the radiolysis of water, such as the hydroxyl radical OH^-, hydrated electron (e_{aq}^-), H atom, hydrogen peroxide (H_2O_2), and hydrogen [1]. Hydrogen peroxide is a strong oxidizing agent and a poison to biological systems, whereas the hydroxyl radical is a strong oxidizing agent and the hydrogen radical is a strong reducing agent. These two radicals can cause several changes in the molecular structure of organic matter [2].

47.1.2 Sources of Ionization Irradiation

There are two classes of ionizing radiation: electromagnetic and particulate. These are γ-rays from radionuclides ^{60}Co or ^{137}Cs, X-rays generated from machine sources operated at or below 5 MeV, and electrons generated from machine sources operated at or below an energy level of 10 MeV [1, 3]. The characteristics of different irradiation sources are summarized in Table 47.1. Although both isotopic and machine sources result in identical impacts on foods, consumers react more favorably to machine sources than isotope sources because of the association of isotopes with the nuclear industry [4]. All three source types require a large plant for economic viability. Much of the high cost is associated with the need for heavy concrete shielding to protect the external environment when the source is in use. In addition, the plant must comply with hygiene and safety legislation relevant to such plants [5].

TABLE 47.1
Characteristics of Irradiation Sources

Radiation Source	Characteristics
Cobalt-60	1. High penetrating power 2. Permanent radioactive source 3. High efficiency 4. Source replenishment needed 5. Low throughput
Electron beams	1. Low penetrating power 2. Switch on–switch off capability 3. High efficiency 4. High throughput 5. Power and cooling needed 6. Technically complex
X-rays	1. High penetrating power 2. Switch on–switch off capability 3. Low efficiency 4. High throughput 5. Power and cooling needed 6. Technically complex

Source: Kilcast [5].

47.1.3 Dose and Dosimetry

The radiation dose (level of treatment) is defined as the quantity of energy absorbed during exposure [6]. Traditionally the dose of ionizing radiation absorbed by irradiated material has been measured in terms of the *rad*, but recently it has been superseded by the *gray* (Gy), which is equal to 100 rad [1]. One gray represents one joule of energy absorbed per kilogram of irradiated product, and the energy absorbed depends on mass, density, and thickness of food [6].

Food irradiation doses are generally characterized as low (less than 1 kGy), medium (1–10 kGy), and high (greater than 10 kGy). Different levels of dose are required to achieve the desired results for products [6]. The energy level used for food irradiation to achieve any technological purpose is normally extremely low, e.g., 0.1 or 1.0 kGy, which would be equivalent to a heat energy of 0.024°C or 0.24°C. The Codex Alimentarius Commission recommended 10 kGy as the maximum energy level or dose of ionizing radiation [7]. At 10 kGy the absorbed energy is equivalent to the heat energy needed to increase the temperature of water by 2.4°C. One gray is equivalent to one joule of energy absorbed per kilogram of material [4]. Loaharanu and Murrell [3] calculated on the basis that 10 kGy of ionizing energy is equivalent to a heat energy of 10 J/g and heat capacity of water is 4.2 J/g °C, i.e., 10/4.2 = 2.4°C. Thus, it is a cold method of food preservation. The absence of noticeable change and small rise in temperature leads to difficulty in detecting whether food has been irradiated [4, 5].

47.1.4 Scope of Irradiation

The potential applications of irradiation are [8, 9] disinfestation, shelf-life extension, decontamination, and product quality improvement.

47.1.4.1 Disinfestation

Disinfestation is one of the important postharvest treatments in food processing, and chemicals are usually used for this purpose. Disinfestation, the control of insects, in fruits can be achieved by doses up to 3 kGy [6]. A low dose of 0.15 to 0.50 kGy can damage insects at various stages of development that might be present on food. Irradiation can damage an insect's sexual viability or capability to become an adult [9].

47.1.4.2 Shelf-Life Extension

One form of shelf-life extension is to inhibit sprouting of potatoes, yams, onions, and garlic at 0.02 to 0.15 kGy [10]. Another form is to delay the ripening and senescence of some

tropical fruits such as bananas, lychees, avocados, papayas, and mangoes at 0.12 to 0.75 kGy [11]. The irradiation also extends the shelf-life of perishable products like beef, poultry, and seafood by decontaminating spoiling microorganisms. Usually, fruits progressively lose their resistance to phytopathogens with ripening. When a low irradiation dose is used to delay ripening, a higher level of resistance is retained in fruits, and microbial development is also delayed as an added benefit [6].

47.1.4.3 Decontamination

Irradiation can reduce microbial load and destruction of pathogens. One form of decontamination could be the use of a low dose (1.0–2.0 kGy) to pasteurize seafood, poultry, and beef. Another form could be a higher dose (3.0 to 20 kGy), such as sterilization of poultry, spices, and seasonings [9].

47.1.4.4 Product Quality Improvement

A higher juice yield could be obtained if fruits are first irradiated at a dose level of several kilograys, thus improving product recovery. Another study showed that the gas-producing factors in soybeans could be markedly decreased with a sequence of soaking, germination, irradiation, and subsequent drying of the beans. This required a dose of 7.5 kGy for maximum effect [12]. It also facilitates reduction of chemicals used in food, such as nitrite and salts. Moreover, irradiation does not leave any chemical residues in the foods [13]. The extent of doses and their purposes are summarized in Table 47.2.

47.1.5 Advantages of Irradiation

Hasegawa and Moy [12] identified at least three distinct benefits of using irradiation to preserve foods. Five advantages of irradiation are discussed next.

47.1.5.1 Minimize Food Losses

Radiation disinfestation and shelf-life extension can reduce the food losses of fresh foods. A great deal of the postharvest losses due to insect infestation can be controlled and minimized by irradiating foods such as grains, pulses, tubers, and fruits. In addition, the shelf life of tubers and some fruits can be extended through sprout inhibition or delayed ripening. Especially in the Third World irradiation has high potential where in many cases food spoils during the postharvest stage [12]. A potential added benefit of the application of irradiation to fruits is the higher juice yield during processing of several commodities [6].

47.1.5.2 Improve Public Health

Foods, especially muscle foods, are contaminated with pathogenic microorganisms or parasites. The decontamination of these fresh foods by irradiation can improve public health concerns. *Salmonella* is a prime source of foodborne illness from poultry products. The use of irradiation up to 3.0 kGy to decontaminate poultry and up to 1.0 kGy to control *Trichinella spiralis* in pork carcasses are approved in the United States [9]. Irradiation is also a method to ensure hygienic quality of solid food [14].

47.1.5.3 Increase International Trade

Many fresh foods are not candidates or not qualified for international trade due to (i) infestation by insects, (ii) infection by microorganisms, and (iii) their limited shelf life, which restricts long-distance shipments. Irradiation can increase or improve the trade of fresh foods over the international market by providing an effective quarantine procedure for infested or infected foods, or helping to prolong the shelf life [9, 14].

47.1.5.4 Alternative to Fumigation of Food

Various chemicals, such as ethylene dibromide, methyl bromide, and ethylene oxide, are used for the fumigation of food and food ingredients. The use of chemical disinfestation treatments is rapidly diminishing due to their toxic nature and environmental impact. For example, the toxic nature of ethylene oxide and the ozone-depleting effect of fumigant ethylene dibromide [5, 14]. Low-dose irradiation of 0.2–0.7 kGy can control insect infestation of grain and other stored products [14]. Irradiation of a dose of 70 Gy led to 99.99% mortality of *Bactrocera invadens* [15].

47.1.5.5 Increase Energy Saving

The energy used for irradiation of food is small compared to canning, refrigeration, and/or frozen storage. The total energy used for refrigerated raw cut-up chicken is 17,760 kJ/kg, for

TABLE 47.2
Extent of Dose and Purpose of Food Irradiation

Dose Limit	Purpose	Dose Limit (kGy)	Examples
Low dose (<1 kGy)	Sprouting inhibition	0.05–0.15	Potatoes, onions, garlic
	Insect and parasite disinfection	0.15–0.50	Cereals, pulses, dried fruit, pork
	Delay of ripening	0.50–1.00	Fresh fruits and vegetables
Medium dose (1–10 kGy)	Reduction of spoilage microorganisms	1.0–3.0	Fish, strawberries
	Reduction of nonspore pathogenic	2.0–7.0	Poultry, shellfish
	Microbial reduction in dry products	7.0–10.0	Herbs, spices
High dose (10–50 kGy)	Sterilization	25–50	Sterile diet meals
Very high dose (10–100 kGy)	Reduces or eliminates virus contamination	10–100	

Sources: Kurstadt [139], WHO [127].

frozen is 46,600 kJ/kg (3–5 weeks frozen storage), and for canned chicken meat is 20,180 kJ/kg. In comparison, refrigerated and irradiated raw cut-up chicken requires a total of 17,860 kJ/kg [16]. Moreover, bans on CFC refrigerants could result in higher costs for refrigerated food in the future, thus combining irradiation and chilling has high potential in the saving of energy during food processing [14]. The reduction of energy requirements can also contribute toward the overall reduction of pollution caused by combustion products of traditional fuels [13].

47.2 EFFECTS ON MICROORGANISMS AND FOOD COMPONENTS

47.2.1 Effects on Microorganisms

Similar to other preservation methods, irradiation affects microbial growth and changes the food components. Ionization irradiation affects microorganisms, such as bacteria, yeasts, and molds, by causing lesions in the genetic material of the cell, effectively preventing it from carrying out the biological processes necessary for its continued existence [17]. Stability of food components also needs to be known to determine their functionality and safety.

47.2.1.1 Mode of Action

The principal targets of irradiation are nucleic acids and membrane lipids. Alteration in membrane lipids, particularly polyunsaturated lipids, leads to perturbation of membranes and to deleterious effects on various membrane functions, such as permeability. The activity of membrane enzymes may be affected as a secondary effect of membrane lipid degradation [6]. Ionization radiations act through changes induced in the DNA structure of the irradiated cells, which result in the prevention of replication or function [3]. The energy levels used are sufficient to disrupt certain bonds in the molecules of DNA, thereby making cell reproduction impossible [4]. Nucleic acids, because of their large size, are the main targets of free radicals generated by irradiation [6]. Chromosomes of bacteria are intrinsically very sensitive and lethal damage occurs as a result of exposure to irradiation. The ability of bacteria to repair a limited amount of such damage gives them considerably greater resistance to such radiations. The efficiency with which different bacteria repair the radiation-induced damage to their DNA varies considerably. The most sensitive vegetative bacteria is *Pseudomonas*, and the most resistant one is *Deinococcus* by a factor of about 100 [1].

47.2.1.2 Level of Dose

Murano [17] reviewed the factors that affect the susceptibility of microorganisms to irradiation, and these are (i) dose level, (ii) temperature, (iii) atmosphere (presence or absence of oxygen), (iv) medium, and (v) type of organism (size, the smaller the target organism, the more resistant it is to ionization radiation; cell-wall characteristics and gram-positive or gram-negative in nature; number and relative age of the cells). In general, the higher the dose applied, the lower the number of survivors and the lower the temperature and the rate of reactions, such as the formation of radicals from water molecules. These radicals can have an indirect effect, by interfering with normal cellular functions such as membrane transport. Table 47.3 shows that minimal doses can achieve significant gains in food safety. If the product is

TABLE 47.3
Effect of Irradiation on Foodborne Parasites

Parasite	Minimum Effective Dose (kGy)	Effect of Irradiation
Protozoa	0.09–0.7	Parasite killed or elimination of infectivity
Toxoplasma gondii	0.251	Killed cyst stage
Entamoeba histolytica		
Trematodes	0.03	Inhibits maturation
Fasciola hepatica	0.15–0.20	Inhibits maturation
Clonorchis sinensis	0.10	Inhibits maturation
Opisthorchis viverrini	0.10	Inhibits maturation
Paragonimus westermani		
Cestodes		
Taenia	>3.00	Complete inactivation of larvae
	0.40	Prevents development in humans
	0.30	Elimination of infectivity
Taenia solium	0.20–0.70	Elimination of infectivity
Echinococcus granulosus	0.50	Elimination of infectivity
Nematodes		
Trichinella spiralis	0.10–0.66	Elimination of infectivity
	0.11	Sterilization of females
Angiostrongylus cantonesis	2.00–4.00	Decreased infectivity
Gnathostoma spinigirum	7.00	Reduced larval penetration
Anisakis species	6.00	Reduced larval penetration

Source: Loaharanu and Murrell [3].

frozen, radical formation is practically inhibited [17]. The *D* value increased from 0.16 kGy at 5°C to 0.32 kGy at –30°C when *Campylobacter jejuni* was inoculated into ground beef [18]. In general, bacteria become more resistant to ionization radiation in the frozen state as well as in the dry state. In both states it is assumed that the contribution of indirect effects from the radiolysis of water is significantly reduced [1]. Off-flavor development in products irradiated in a dry state is less than that of moist products. This is due to the low formation of free radicals at reduced moisture content [2]. The irradiation atmosphere may have an effect, but this may only occur under specific conditions [17]. The composition of the irradiating medium will affect the resistance of microorganisms to irradiation. As a general rule, the simpler the life form, the more resistant it is to the effects of irradiation. For instance, viruses are more resistant than bacteria, which are more resistant than molds, which are more resistant than human beings. Also, within bacteria, some genera are found to be more resistant than others [17]. Bacterial spores are more resistant than their corresponding vegetative cells by a factor of about 5 to 15 [19].

The effectiveness of irradiation to control the infectivity of foodborne parasites is summarized in Table 47.3 from Reference [3]. Low-dose irradiation (below 1 kGy) offers a unique opportunity for controlling the infectivity of a number of foodborne parasites without changing the character of the food. Among the groups of foodborne parasites, trematodes appear to be the most sensitive to irradiation, followed by cestodes and protozoa [3]. The *D* values of various foodborne pathogens in fresh meat are given in Table 47.4.

47.2.2 Effects of Irradiation on Food Components

In addition to microbial growth, the effects of irradiation on other nutritional components need to be identified before using the technology. The effects of irradiation on nutritional qualities of foods are reviewed by Graham [2]

47.2.2.1 Effects on Proteins

Low doses of irradiation may cause molecular uncoiling, coagulation, unfolding, and even molecular cleavage and splitting of amino acids. Apparently, peptide linkages were not attacked, and the main effects were concentrated around sulfur linkages to hydrogen bonds [2]. The sequence of protein bonds attacked by ionizing radiation as follows: -S-CH_3, -SH, imidazole, indole, alpha-amino, peptide, and proline [20]. At 10 kGy radiation, an overall increase in total free amino acids was observed mainly due to the rise in the levels of glycine, valine, methionine, lysine, isoleucine, leucine, tyrosine, and phenylalanine [2]. Irradiation is thought to bring about unfolding of the protein molecule, leading to the availability of more reaction sites [2].

Irradiation also affects the functional properties of proteins. In the case of egg, the doses required for effective *Salmonella* reduction give undesirable side effects, such as loss of viscosity in the white and off-flavors in the yolk [5]. An egg irradiated with 6 kGy showed a thin, watery condition that may be due to the destruction or alteration of ovomucin, the main thickening compound of egg albumin. The casein in milk resulted in an increase in rennet coagulation time and reduced heat stability [2].

The off-flavor development at high doses is due to the presence of benzene, phenols, and sulfur compounds formed

TABLE 47.4
Susceptibility of Various Foodborne Pathogens to Irradiation in Fresh Meat

Organism	T (°C)	Product	D_{10} value (kGy)
Listeria	—	Meat	0.40–0.60
Salmonella	—		0.40–0.50
Escherichia coli O157:H7	—		0.25–0.35
Campylobacter	—		0.14–0.32
Yersinia	—		0.14–0.21
Aeromonas	—		0.14–0.19
Staphylococcus aureus	5	Turkey breast meat	0.45
Campylobacter jejuni	30	Ground turkey	0.16
	5		0.19
	–30		0.29
Salmonella heidelberg	0	Poultry (air packed)	0.24
	0	Poultry (vacuum packed)	0.39
Salmonella enteriditis	5	Egg powder	0.60
	3	Ground beef	0.55–0.78
Salmonella spp.	5	Turkey breast meat	0.71
Listeria monocytogenes	5	Beef	0.45
Escherichia coli O157:H7	5	Ground beef patties	0.27–0.38

Source: Radomyski et al. [140], Smith and Pillai [128].

from phenylalanine, tyrosine, and methionine, respectively. Flavor changes and off-flavors resembling a burnt flavor was observed in the irradiated milk [2]. Irradiation of cheese usually produces smoky off-flavors. Irradiation of soft cheeses at a dose of 1–2 kGy is sufficient to reduce food pathogens and does not impair flavor quality, thus careful dose application is certainly key for off-flavor components [5].

47.2.2.2 Effects on Carbohydrates

Irradiation can break high molecular weight carbohydrates into smaller units leading to depolymerization. This process is responsible for the softening of fruits and vegetables through breakdown of cell wall materials, such as pectin. However, softening may have advantages or disadvantages depending on the requirement. For example, it may be advantageous for juice yield and reduced drying and cooking times of dehydrated products [5]. Sugars may be hydrolyzed or oxidized when subjected to gamma irradiation [6]. The irradiation of wheat at 0.2–10 kGy increased the initial levels of water-soluble reducing sugars by 5–92% compared to untreated samples [21]. Such an overall increase in initial total reducing sugars resulted from the stepwise and random degradation of starch. These changes are highly advantageous in the generation of bread flavor and aroma by reducing sugar–amino acid reactions [2]. Irradiation of pure carbohydrates produced degradation products that have mutagenic and cytotoxic effects. However, these undesirable effects were produced using very high doses of irradiation [2].

47.2.2.3 Effects on Lipids

Irradiation initiates the normal process of autooxidation of fats, which gives rise to rancid off-flavors. Highly unsaturated fats are more readily oxidized than less unsaturated fats. This process can be slowed by the elimination of oxygen by vacuum or modified atmosphere [5]. In lipids, particularly unsaturated fatty acids, radiolytic decomposition is via a preferential break at the level of the carbonyl function of the double bond [22]. This decomposition induces the formation of some volatile compounds responsible for off-odors [23]. The formation of peroxides, volatile compounds, and development of rancidity and off-flavors have been reported [24, 25]. The peroxide formed can also affect certain labile vitamins, such as vitamins E and K [2].

The lipids in cereals degraded only at high doses of irradiation, and no significant effects on iodine value, acidity, or color intensity of wheat flour lipids were observed. At 10 kGy, a 20% increase in total free lipids and a 46% decrease in bound lipids were observed [26]. Lipid–protein complexes, critical in baking, were not noticeably affected at low doses up to 2 kGy.

The volatile oil content of spices has a dose-dependent reduction effect in black pepper [27] and ginger [28] above 6 kGy. A similar reduction was also observed in the case of Ashanti pepper berries when 47 essential oil compounds were analyzed individually at a dose of 10 kGy [29].

47.2.2.4 Effects on Vitamins

The extent of vitamins C, E, and K destruction depends on the dosage used, and thiamine is very labile to irradiation. The losses are low with low dose. Ascorbic acid in solution is quite labile to irradiation, but in fruits and vegetables seems quite stable at low doses of treatment [2]. Vitamins, particularly those with antioxidant activity, such as A, B_{12}, C, E, K, and thiamine, are degraded when irradiation is carried out in the presence of oxygen [6].

Irradiation can also partially damage vitamin C and B_1. Kilcast [5] mentioned that literature referring to vitamin loss is misleading in many cases. Vitamin losses are often quoted at unrealistically high irradiation doses or under unrealistic conditions. In particular, vitamin C loss is often equated with ascorbic acid loss, ignoring the fact that irradiation converts ascorbic acid into dehydroascorbic acid, which is also active as a vitamin [5, 6].

47.2.2.5 Effects on Enzymes

Enzymes in foods must be inactivated prior to irradiation because they are much more radiation-resistant than microorganisms. Usually, enzyme inactivation is accomplished thermally. Generally, it may be said that complete inactivation of enzymes requires about 5 to 10 times the dose required for the destruction of microorganisms [2]. The *D* values of enzymes can be 50 kGy and almost four *D* values would be required to complete destruction [20]. Thus, irradiated foods can be unstable during storage due to their susceptibility to enzymatic attack than nonirradiated foods [2]. High resistance of enzymes to irradiation has been demonstrated with milk phosphatase, which was not destroyed by irradiation doses sufficient to sterilize milk [30]. Enzymes are affected by the indirect effects of the formation of free radicals in the solvent phase, thus dilute solutions of enzymes are relatively more sensitive to irradiation than are concentrated solutions. Moreover, enzymes in their natural environments, as in foods, are relatively very resistant [2]. The activity of enzymes is unaffected at normal doses and thus it limits the achievable shelf-life extension of fruits and vegetables [5].

47.3 APPLICATIONS OF IRRADIATION IN FOODS

47.3.1 Plant Foods

Plant tissues showed a transient increase in respiration and ethylene production even at low doses. The rate of respiration increased linearly with increasing dose of irradiation. The transient rate of respiration decreased back to preirradiated levels within 24 h for 0.3 kGy, but slower with an increasing dose. Ethylene production also increased after irradiation and it reached a maximum at 1 kGy. It has been suggested that irradiation beyond 1 kGy caused membrane damage since ethylene production is membrane-associated [6]. There was a shift from glycolysis toward the pentose phosphate shunt in bananas and toward the glyoxylate cycle in bananas and

mangoes [31]. At higher doses, climacteric fruits may not ripen normally and may develop uneven coloring and skin discoloration [6].

Fruits suffer physiological disorders when exposed beyond their limits of tolerance. These undesirable symptoms are mainly tissue softening and enzymatic browning [32]. Tissue softening is caused by (i) partial depolymerization of cell wall polysaccharides, mainly cellulose and pectins [33]; and (ii) damage to the cell membrane [34]. Enzymatic browning is an indication of cell decompartmentation due to damage of membranes, thus bringing phenolic substrates in contact with polyphenoloxidases [35]. The damage to the cell membrane may result in (i) loss of intracellular water, (ii) cell turgescence, and (iii) oxidative attack on polyunsaturated fatty acids of membrane lipids [6]. The oxidation can be minimized by irradiating in an atmosphere with reduced oxygen content, but treatment efficiency is reduced. Voisine et al. [36] mentioned that a high carbon dioxide atmosphere was shown to protect tissues from irradiation- induced loss of membrane proteins. Thus, low-dose irradiation combined with a modified atmosphere is increasingly considered for control of microorganisms and delayed ripening. Couture and Willemot [37] showed the synergistic action of irradiation combined with high carbon dioxide for control of mold development on strawberries. A decay incidence of *Rhizopus stolonifer* and *Botrytis cinerea* on strawberries packaged under 7% oxygen and 20% carbon dioxide and irradiated at 1 kGy was reported [38]. Table 47.5 shows the response of a number of fruits to irradiation. The applications of irradiation in specific plant materials are discussed next.

47.3.1.1 Spices

There is an increasingly important use of irradiation for decontamination of spices. Spices imported into Western Europe are often heavily contaminated by pathogenic microorganisms as a consequence of open air drying procedures [5]. The prevalent microorganisms of pepper are *Clostridium*, *Staphylococcus*, *Bacillus*, *Aspergillus*, and *Fusarium* species. Doses of 2.5 kGy reduced the fungal and bacterial load by 2 log cycles and 7.5 kGy eliminated the fungal population of ground or whole pepper.

Yang et al. [39] found that the treatment of garlic bulbs with 0.15 kGy can inhibit sprouting and reduce weight loss during storage. The irradiation affects the flavor compounds of garlic. Kwon and Yoon [40] reported that the content of diallyl disulfide in garlic was slightly reduced by irradiation of 0.05–0.10 kGy. There was no difference in the contents of diallyl disulfide upon comparison of 0.05 kGy irradiated with unirradiated bulbs during storage [41]. The irradiated bulbs showed no difference in odor during storage when compared with untreated [42]. The dose of 0.15 kGy affected the volatile compounds in garlic bulbs during storage at room temperature [43]. The content of diallyl disulfide decreased immediately after irradiation. At the end of 8 months of storage both irradiated and untreated samples showed a significant increase in diallyl disulfide. The quantities of some major volatiles were significantly decreased in irradiated ginger rhizome after 3 months of storage when 0.05 kGy irradiation was applied [44].

47.3.1.2 Fruits and Vegetables

47.3.1.2.1 Berries

The postharvest shelf life of cherries, blueberries, and cranberries can be extended at low doses of irradiation [45]. Blueberries irradiated at 0.25, 0.5, 0.75, or 1.0 kGy can be stored at 1°C for 1, 3, and 7 days, and 2 additional days at 15°C, respectively, [46]. The firmness of Sharpblue blueberries was slightly affected by dose, but firmness of Climax blueberries was not affected by irradiation. Flavor and texture were negatively affected as dose increased for berries of both cultivars. Weight loss, decay, peel color, total soluble solids, and titratable acidity were not affected by dosage. Irradiation at or below 0.75 kGy was determined not to be detrimental to the postharvest quality. This treatment can be an effective alternative quarantine treatment to methyl bromide [46].

47.3.1.2.2 Mangoes

Mango preservation would greatly benefit from irradiation treatment. The effects of irradiation depend on the degree of maturity [6]. The optimal dose was 0.75 kGy for three-quarter ripe fruits at room temperature [11]. Combined with a mild heat treatment by hot water dip or vapor for 5 min at 55–55°C yielded even better results [47]. The surface scalding is a limiting factor,

TABLE 47.5
Response of 23 Fruits to Irradiation

Effect	Results	Materials
Beneficial	Ripening delayed	Bananas, mangoes, papayas
	Senescence delayed	Sweet cherries, apricots, papayas
	Storage decay controlled	Tomatoes, strawberries, figs
Not beneficial	Lack of tolerance	Pears, avocadoes, lemons, grapefruits, oranges, tangerines,
	Ripening accelerated	cucumbers, summer squash, bell peppers, olives, plums, apples, table grapes, cantaloupes
		Peaches, nectarines
	Irradiation tolerance only	Pineapples, lychees, honeydew melons

Source: Akamine and Moy [11].

since at 0.25 kGy scalding occurred on the mature green fruits, and tolerance increased with maturity [11].

47.3.1.2.3 Carrots

Doses up to about 0.1 kGy had little effect on the firmness of apples, carrots, and beets, but rapid softening occurred at higher doses [48, 49]. The effective range for control of rotting and sprouting is 0.1–1 kGy, which means substantial softening can occur [50]. Bourne [50] studied the kinetics of softening of carrot with doses from 0 to 50 kGy. Two distinct regions were observed: a steep negative slope for doses up to 15 kGy and a shallow negative slope beyond 15 kGy. A two-stage softening rate curve is qualitatively similar to that for thermal softening of carrot.

47.3.1.2.4 Papaya

Papaya can tolerate up to 1 kGy γ-radiation before surface scald occurs. The surface color development is not disrupted by up to 2 kGy, flavor and aroma up to 4 kGy, and tissue breakdown up to 5 kGy [11, 51]. A dose of 0.75 kGy was considered the optimum dose for retention of fruit firmness with only slight reduction of storage decay [51]. A hot water dip at 48.9°C for 20 min in combination delayed ripening with optimum dose of 0.75 kGy [11, 47]. A hot water dip alone accelerated ripening, while irradiation alone provided only slight control of decay, and a hot water dip was preferable to vapor heat treatment [6]. The respiratory activity was initially elevated immediately after irradiation and then returning to the level of untreated fruit within 24 h [52]. Paull [53] studied the effect of 0.25 kGy on papaya and found irradiated fruit softened more uniformly than nonirradiated fruit.

Zhao et al. [54] found irradiation had no effect on the skin and flesh color development of ripening papaya. Irradiation induced immediate depolymerization and demethoxylation of papaya pectic substances indicated by an increase in water-soluble pectin, and decrease in chelator-soluble and alkali-soluble pectin with a significant decline in methanol content. The linear decrease in firmness up to 1.5 kGy was parallel to the change of pectin fractions. Pectin methylesterase was not affected either immediately after irradiation or during ripening, by irradiation at doses up to 1.5 kGy.

47.3.1.2.5 Strawberries

Irradiation at doses of 1, 2, and 3 kGy effectively prolonged the shelf life of strawberries stored at 4°C for 5, 13, and 16 days, respectively [55]. Maxie and Abdel-Kader [56] indicated that strawberries may tolerate an irradiation dose up to 2 kGy for reducing fungal infection without quality changes. The softening of strawberries occurred after irradiation [31, 57, 58]. The softer texture above 2 kGy may limit use of higher doses. The firmness of strawberries decreased as irradiation doses increased 0.5, 1, and 2 kGy [59, 60]. Success depends on cultivar: the firmer Tioga strawberries tolerated radiation better than the softer Brighton strawberries [47]. Several studies indicated that irradiation-induced texture change was associated with changes in pectic substances [49, 61, 62]. Water-soluble pectin increased and oxalate-soluble pectin decreased at 0 and 1 day after 1 and 2 kGy irradiation. Fruit firmness correlated with oxalate soluble pectin content. Total pectin and nonextractable pectin were not affected by irradiation. The oxalate-soluble pectin content and firmness of irradiated strawberries increased slightly at the beginning of 2°C storage and then decreased as storage time increased [60]. Irradiation enhanced the sweetness of strawberries by reducing titratable acidity in comparison with untreated sample [63]. The depolymerization of carbohydrate polymers, such as starch and cellulose, may slightly increase the sugar content [33].

47.3.1.3 Cereals and Grains

Grains and cereals are treated with low doses of irradiation to eliminate fungi since some of these organisms can produce mycotoxins [2]. Irradiation doses in the range of 0.2–1.0 kGy are effective in controlling insect infestation in cereals [64]. Increasing the dose to 5 kGy totally kills the spores of many fungi surviving the lower doses [65]. In addition to its protective role from insects and microorganisms, irradiation also affects various quality criteria of cereal grains [66]. The amylograph peak viscosity and falling number values of flour decreased with the increasing irradiation dose [67, 68]. Rao et al. [69] also found that amylograph peak height and dough stability decreased with the increasing dose. At 10 kGy, loaf volume and crumb grain were impaired. The overall bread quality of wheat was greatly reduced at a medium dose of irradiation 1–10 kGy [70]. Loaf volume and baking quality deteriorated above 5 kGy irradiation irrespective of the baking formula [71].

Increased cooking losses and inferior scores in sensory were observed in Japanese noodles when wheat was irradiated in the range of 0.2 to 1.0 kGy [72, 73]. Koksel et al. [66] studied the effects of irradiation up to 5.0 kGy on durum wheat and semolina properties and spaghetti cooking quality. The falling number and sedimentation values of wheat meals decreased with increasing dose levels. This indicated the alterations in both starch and gluten components. Irradiation also caused important changes in the spaghetti cooking properties. Total organic matter and solid substance lost values of both cultivars increased with irradiation. Koksel et al. [66] mentioned that irradiation may be useful at 1 kGy dose for treatment of grain for insect control without adversely affecting quality. Above 1 kGy dose, irradiated samples exhibited lower scores for stickiness, firmness, and bulkiness compared to unirradiated samples due to deterioration in both starch and gluten. Cowpeas can be preserved in polyethylene bags (100 μm) after ionizing treatment at doses of less than 0.10 kGy without causing unfavorable nutritional consequences [74].

47.3.2 Animal Foods

In 1997, the U.S. Food and Drug Administration (FDA) approved the use of ionizing radiation to inactivate pathogenic bacteria in red meat [75]. The irradiation is effective in preventing or delaying the microbial spoilage of fresh meats and poultry. As Mitchell [4] mentioned, early studies indicated that irradiation at doses between 0.25 and 1 kGy under aerobic

conditions increased microbiological shelf life but accelerated rancidity [76]. A tallowy odor and flavor developed during storage. The fat was noticeably bleached, and peroxide accumulated more rapidly in the irradiated than in the control fat. In the case of meats, doses up to 2.5 kGy control *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli* in poultry and other meats. The doses in excess of 2.5 kGy may change flavor, odor, and color, but these changes can be minimized by irradiating at low temperature or in the absence of oxygen [4].

Irradiation treatment is not effective to stop the changes in meats that adversely affect consumer acceptance, such as oxidation of pigment to yield brown or gray discolorations by atmospheric oxygen; drip loss from the cut surface of lean meat; and oxidation of meat lipids, which causes off-flavors by atmospheric oxygen [4]. Thus, irradiation coupled with vacuum packaging has the potential to extend the shelf life [4]. Table 47.6 shows the threshold dose for an identifiable irradiation flavor in meats.

Hydrogen generated during the irradiation of frozen meats is a promising marker for distinguishing between irradiated and unirradiated frozen food [77]. Poultry meat in particular is known to be susceptible to color changes following irradiation [78]. A pink color is produced in fresh poultry when it is treated with irradiation [79]. Irradiated chicken breasts were found to exhibit increased greater redness (a* values) when compared with the unirradiated controls [80].

47.3.2.1 Poultry

At a low dose of radiation, not all microorganisms are destroyed and survivors such as *Moraxella*, *Acinetobacter*, *Lactobacillus*, and *Streptococcus* can cause spoilage [81]. A mixed microflora to a predominant gram-positive microflora usually exists during the postirradiation stage [82]. Doses of 2–2.5 kGy are effective in controlling *Listeria* [83, 84] and a dose of 1.0–2.5 kGy is adequate to eliminate *Pseudomonas aeruginosa*, and 2.5–5.0 kGy to eliminate *Serratia marcescens* [85]. A dose of 1.50 kGy was effective for *Staphylococcus aureus* in deboned chicken when irradiated in vacuum at 0°C and held at 35°C for 20 h [86]. In the case of *Escherichia coli* in deboned chicken meat, a 90% decrease of viable cells can be achieved by doses of 0.27 kGy at 5°C and 0.42 kGy at –5°C [87].

TABLE 47.6
Threshold Dose for an Identifiable Irradiated Flavor for Meats

Meat	Temperature (°C)	Threshold (kGy)	Reference
Pork	5–10	1.75	Sudarmadji Urbain [141]
Beef	5–10	2.50	Sudarmadji Urbain [141]
Chicken	5–10	2.50	Sudarmadji Urbain [141]
Lamb	5–10	6.25	Sudarmadji Urbain [141]
Lamb	—	2.40	Dempster [112]

In the Netherlands, a maximum dose of 3 kGy is permitted on an unconditional basis in case of poultry irradiation [88], and in Israel and South Africa, a dose of 7 kGy is allowed to eliminate pathogenic bacteria [89]. Mulder [82] recommended a dose of 2.5–5 kGy since this can extend shelf life at chill temperatures from 6 days up to 14 days without insignificant organoleptic quality change. However, doses as low as 0.5 kGy can induce a radiation odor and a 2.5 kGy can induce flavor changes, which may be removed on subsequent cooking [82, 85]. Table 47.7 shows the changes in odor of irradiated chicken carcasses during storage at 1.6°C.

The color of meat depends on three factors: concentration of heme pigments, the chemical state of these pigments, and the physical light scattering properties of the meat structure [90]. Patterson [81] studied the sensitivity of irradiation under air, carbon dioxide, vacuum, and nitrogen on seven bacterial species inoculated onto sterile poultry meat. *Streptococcus faecalis* and *Staphylococcus aureus* were not sensitive to atmosphere, and others such as *Pseudomonas putida*, *Salmonella typhimurium*, *Escherichia coli*, *Moraxella phenylpyruvica*, and *Lactobacillus* were more sensitive in atmospheres other than air. In general, a vacuum or carbon dioxide atmosphere during irradiation had the most lethal effect, and bacteria may be more resistant to irradiation if packaged under nitrogen than carbon dioxide or air [4, 81].

47.3.2.2 Mutton Lamb

Irradiation of vacuum-packaged mutton backstraps at 4 kGy prevented the growth of bacteria for at least 8 weeks at 0–1°C [91], while *Brochothrix thermosphacta* and gram-negative bacteria grew on telescoped lamb carcasses irradiated at 2.4 kGy and stored at 5°C. The total population did not exceed 10^5 cfu/cm^2 during 16 weeks storage [92]. However, at these high doses, adverse effects on sensory attributes and increased volume of weep released were observed. Meat chunks irradiated at 1.0 kGy and 2.5 kGy were acceptable for 3 and 5 weeks, respectively, whereas for minced meat it was 2 and 4 weeks [93]. In contrast, unirradiated meat chunks and mince spoiled within 1 week of storage at 0–3°C [4].

47.3.2.3 Beef

Pseudomonas, Enterobacteriaceae, and *Brochothrix thermosphacta* were strongly inhibited beef meat on irradiated samples and sensory properties were not altered [4]. Rodriguez et al. [94] studied the effect of 2 kGy irradiation on fresh top round beef, packed aerobically in polyethylene film. Psychrotroph counts on the untreated samples reached 10^7 cfu/cm^2 after storage between 8 and 11 days, while similar counts were not observed in irradiated samples until 28 days of storage. The shelf life of vacuum-packaged raw meat can be extended considerably at doses of 1–5 kGy, which also yielded satisfactory sensory quality [95, 96]. Grant et al. [97] studied the effect of 2 kGy irradiation on growth and toxin production by *Staphylococcus aureus* and *Bacillus cereus* on roast beef. Irradiation resulted in a 3–4 log reduction in the numbers of both pathogens. Toxin production by both pathogens was also delayed by irradiation. Roast beef and gravy samples

TABLE 47.7
Changes in Odor of Irradiated and Unirradiated Chicken Carcasses Stored at 1.6°C

Storage Time (Days)	Unirradiated	Irradiated	
		2.5 kGy	5.0kGy
0	Fresh chicken	Slight irradiation odor	Irradiation odor
4	Fresh chicken	Fresh chicken odor	Slight irradiation odor
8	No odor	Fresh chicken odor	Fresh chicken odor
11	Slight off-odor	Chicken odor	Chicken odor
15	Putrid	Slight chicken odor	Slight chicken odor
18	Putrid	Stale chicken odor	Slight chicken odor
22	Putrid	Stale chicken odor	Slight chicken odor
31	Putrid	Stale chicken (sour)	Slight chicken odor

Source: Kahan and Howker [142].

irradiated at 2 kGy and stored at 5°C and 10°C showed similar growth rates of *Listeria monocytogenes*, with a lag period of 6–9 and 2–4 days, respectively, compared to 1–2 days and less than 0.1 days in nonirradiated samples [98].

Postmortem aging of beef is typically done by holding carcasses or cuts between –1°C and 4°C for up to 3 weeks. During that time, tenderness improves and a distinctive flavor develops. Snyder [99] suggested that carcass aging at high temperature followed by irradiation can reduce microbial numbers. Irradiation in conjunction with modified atmosphere packaging containing 25% carbon dioxide and 75% nitrogen could be used for an accelerated aging process of beef at 30°C for 2 days [100]. Their result was based on tenderness, chemical, visual, and microbiological effects. Moreover, if irradiated beef were chilled immediately after aging, this process could improve tenderness without excessive microbial growth and would be more efficient than aging carcasses at high temperatures followed by irradiation [100].

In a summary by Sommers [75]:

- Irradiation can inactivate pathogenic bacteria occasionally found in ground beef, such as *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes*.
- Irradiation does not make food radioactive. (iii) Irradiation, when used appropriately, does not change the aroma, taste, aftertaste, texture, or overall liking of ground beef, including frozen ground beef supplied as part of the U.S. National School Lunch Program.
- There is no detectable increase in the risk of cancer associated with long-term consumption of radiation pasteurized meat as determined by multispecies, multigeneration feeding studies conducted in animals.
- Irradiated ground beef is nutritious and wholesome.
- Irradiation is only effective as part of a comprehensive program designed to improve the microbiological safety of ground beef, not to clean up unacceptable product.

47.3.2.4 Pork

Sivinski and Switzer [101] mentioned that a low-dose irradiation treatment between 0.30 and 1.0 kGy may be used to inactivate the parasite *Trichinella spiralis* in pork. The effect of 1 kGy irradiation on vacuum-packaged pork stored up to 21 days at 4°C reduced the numbers of mesophiles, psychrotrophs, anaerobic bacteria, and Staphylococci throughout storage. The effects of irradiation on sensory characteristics of pork loin were minimal. Irradiation of pork striploins (vacuum packaged, pH 6.2–6.6) at 2.5 and 4.3 kGy reduced by 3 and 5 logs in the number of viable bacteria present [102]. Significant organoleptic (color, odor, and flavor) changes occurred up to 1.0 kGy. Shelf life increased from 8 to 11.5 days for vacuum-packaged ground pork irradiated at 1 kGy when stored at 5°C [103, 104], while from 4 to 6 weeks at 0°C [102]. The storage life of vacuum-packaged pork at 0°C and 5°C was doubled by 2.5 and 4.3 kGy treatment, but produced undesirable side effects of changes in color and odor of uncooked meat [105]. The odor resulting from a dose of 2.5 kGy was sufficient to make the meat unacceptable. Treatment using a dose of 1.0 kGy was effective in extending storage life and produced only slight changes in color and odor [105]. After 12 days of refrigerated storage, *Lactobacillus* and coryneform bacteria predominated in the irradiated meat [103]. The microbial shelf life of vacuum packaged pork lions stored at 2°C was extended from 41 days to 90 days when treated by 3 kGy [106]. Samples of vacuum-packaged ground fresh pork were irradiated at doses from 0.57 to 7.25 kGy and stored at 2°C for 35 days and analyzed. Surviving microflora were not detected in any sample that received an absorbed dose of 1.91 kGy or higher. *Staphylococcus*, *Micrococcus*, and yeast

species predominated in samples that received a dose of 0.57 kGy [107]. There is no significant difference between irradiated samples and controls on lipid oxidation of irradiated pork chops during storage [108].

After reviewing the literature on irradiation in combination with modified atmosphere packaging, Mitchell [4] mentioned that substantial extensions in sensory shelf life can be achieved using doses from 0.5 to 1.75 kGy and modified atmospheres with 25% to 50% carbon dioxide (balance nitrogen). The product must be stored at 5°C or less to achieve extended shelf life. Irradiation in the presence of oxygen has a detrimental effect on chemical and sensory characteristics, resulting in rejection of the product [18, 109]. A list of shelf life of irradiated and unirradiated pork loin in 100% nitrogen is given in Table 47.8. The shelf life can be different depending on the quality attributes, such as microbial and color. The microflora of irradiated modified atmosphere packaging of pork is almost exclusively lactic acid bacteria [110]. Inoculation studies showed that *Clostridium perfringens* was the most resistant and *Yersinia enterocolitica* the most sensitive of the pathogens *Clostridium perfringens*, *Yersinia enterocolitica*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhimurium* [111].

47.3.2.5 Processed Meats

The amount of nitrite required in cured meats possibly can be reduced by irradiation, thus the chance of nitrosamine formation can be lowered [95, 112]. The nitrite levels in irradiated bacon can be reduced from normal levels of 120–150 mg/kg to 20–40 mg/kg without loss of organoleptic quality [113]. Moreover, the load of spores in additives, such as herbs and spices frequently used in meat products need higher doses of radiation [114]. Wills et al. [6] treated vacuum-packaged sliced corn beef with radiation doses of 1, 2, and 4 kGy. The initial microbial load reduced by 1, 2.5, and 5 log cycles; however, slight changes in aroma and flavor at 2 kGy were observed and storage life was doubled to about 5 weeks. Enterobacteriaceae was effectively inactivated by irradiation with doses of 1 or 2 kGy when sensory effects were minimal. The product can be stored up to 5–7 days when treated at 2 kGy. Ground beef patties irradiated at 2.0 kGy under vacuum remained unspoiled even after 60 days of refrigerated storage [115]. In the case of minced meat, a slight reduction of pH to 5.2–5.3 was observed on vacuum-packed products at a dose of 2 kGy. Lactic acid bacteria were more resistant to radiation and became the dominant species during storage. The combination of pH reduction and irradiation prevented growth of Enterobacteriaceae even at 10°C incubation [116].

The sensory characteristics (flavor, texture, juiciness, and aftertaste) of ground beef patties irradiated at 2.0 kGy and stored under refrigerated conditions were studied by Murano et al. [115]. After one day, irradiated patties were significantly more juicy and tender than nonirradiated patties, while after 7 days no significant difference was observed.

TABLE 47.8
Shelf Life of Irradiated and Unirradiated Pork Loin in 100% Nitrogen

Irradiation Dose (kGy)	Storage Temperature (°C)	Shelf Life (Days)			
		Microbial	Color	Odor	Overall
0	5	14	9	16	9
1	5	21	35	26	21
0	25	2	<2	<2	<2
1	25	10	>14	2	2

Source: Lambert et al. [143].

47.3.2.6 Fish and Fish Products

Singh [117] and Nickerson et al. [118] reviewed the irradiation of meats and fish and their shelf life. The control of pathogenic organisms and the extension of shelf life of fresh fish can be achieved with relatively low doses less or equal to 2.5 kGy [117]. However, *Clostridium botulinum* (A, B, E, and F) present in fish and fish products remained unaffected by the low doses of irradiation. Thus, precautions to storage under 3°C and making oxygen available to the product need to be taken. In the case of dried fish (moisture <20%), a dose of 0.3 kGy is sufficient to control insect attack and their larvae [119], but at higher levels of moisture from 20% to 40%, a dose of 0.5 kGy is required.

Mold growth can also contribute to spoilage depending on the moisture level in the fish. Control of the mold growth by irradiation alone requires doses of 3 to 5 kGy [119]. Bacterial spoilage is also a problem in semidry and fresh fish, and fish products [117]. Typical doses up to 2.5 kGy are generally adequate to control the spoilage bacteria and extend the shelf life of fresh fish. The optimum irradiation doses and shelf lives of freshwater and marine fish, and shellfish were compiled by Singh [117]. In general, the shelf-life extension on irradiation is dependent on the conditions of irradiation and storage, and seem to vary from species to species of fish.

Al-Kahtani et al. [120] studied the effects of gamma irradiation (1.5–10 kGy) and postirradiation storage up to 20 days at 2°C on tilapia and Spanish mackerel. They found that (i) total volatile basic nitrogen formation was lower in irradiated fish than in the unirradiated; (ii) a larger increase in thiobarbituric acid values continued gradually during storage; (iii) some fatty acids ($C_{14:0}$, $C_{16:0}$, and $C_{16:1}$) decreased upon irradiation, while others ($C_{18:0}$, $C_{18:1}$, and $C_{18:2}$) increased; (iv) thiamin loss was more severe at higher doses ≤4.5, whereas riboflavin was not affected; and (v) doses higher than 3.0 kGy caused a decrease in alpha and gamma tocopherols.

Chen et al. [121] studied low doses (2 kGy or less) in reducing pathogenic and spoilage microorganisms, and the sensory quality of crab products (white lump, claw, and fingers) through 14 days of ice storage. Irradiation effectively reduced spoilage bacteria extending shelf life by more than 3 days beyond control samples. Fresh crab odor and flavor were similar for treated and control samples, while off-flavors and odors developed more rapidly in controls during storage.

Overall, acceptability scores for irradiated crab samples were higher than for control samples through 14 days ice storage.

47.4 TECHNOLOGICAL PROBLEMS AND LIMITATIONS OF IRRADIATION

47.4.1 Major Problems of Irradiation

47.4.1.1 Investment Cost

Successful implementation of a new technology depends on the availability of a proper infrastructure within a given country [14]. Irradiation has high capital costs and requires a critical minimum capacity and product volume for economic operation [13, 122], although irradiation has a low operating cost and requires low energy [123].

47.4.1.2 Risk–Benefit Analysis of Dose

There are threshold doses above which organoleptic changes and off-flavors development occur. But at low doses, all microorganisms and their toxins will not be eliminated. Willemoti et al. [6] mentioned that variability of the effects leads to difficulty in standardizing irradiation treatment. The success of treatment depends on commodity and cultivar, dose of radiation, degree of maturity, physiological status of fruits, temperature and atmosphere during and after treatment, pre- and postharvest treatments, and susceptibility of the microorganisms to be controlled [6]. Tolerance changes with the degree of maturity. Physiological status, such as mechanical injury, season, and humidity at time of harvest, also plays a role. The response of each individual batch of fruits is therefore difficult to predict, thus generalized dose levels and their consequences in quality are difficult to develop.

47.4.1.3 Damage of Packaging Materials

The packaging materials used during irradiation should not cause the production and release of undesired substances that may migrate into the food. Irradiation may affect different packaging materials in different ways. At doses of 60 kGy and higher, some damage may occur in tin-coated steel and aluminum containers, but at the level of sterilizing doses there should not be any affect. The enamels usually used in the interior must also be of the proper type. For example, for high-fat-content foods, oleoresin enamels are unsuitable but are suitable for enzyme inactivation of foods. Irradiation apparently depolymerizes butyl-rubber sealing compounds used in cans [2]. The shape of the container is also very important. A cubical form is the most satisfactory for optimum dose distribution during irradiation [2].

The effect of irradiation on plastic films depends on the nature of the packaging film, layers of packaging film and additives in formulation, temperature and oxygen content during treatment, treatment dose, dose actually absorbed, and contact with foods [124]. At doses less than 20 kGy, physical changes in flexible containers are negligible. High doses above 30 kGy cause brittleness in cellophanes, saran, and plioform, while 20 kGy or more can cause inconsequential physical changes in mylar, polyethane, vinyl, and polyethylene plastic films [2]. The physical damages can be considerably reduced by the addition of aromatic additives. At strong doses at 50 kGy, mechanical properties of polymers can be improved by cross-linking [125]. At the doses generally applied from 3 to 25 kGy, the migration of water increases [123]. Properties of polyethylene terephthalate (PET) are well preserved during irradiation [126]. Table 47.9 shows the FDA-approved levels of irradiation dose for different packaging materials.

An important problem in the irradiation of foods in plastic containers is the production of gas and volatile compounds, which may migrate to the food and cause off-flavors. At sterilizing doses, nylon gives rise to little off-odor production, whereas in the case of polyethylene, short fragmentations of the polymer are produced and enter the food [2]. Some food packaging materials produce volatile compounds under certain conditions. Volatile compounds are formed in polyethylene, polyester terephthalate, and oriented polypropylene after irradiation dose from 5 to 50 kGy. Twenty-two compounds were identified for polyester terephthalate; 40 for oriented polypropylene; and only acetone was identified for polyethylene, which can be good candidate for irradiation of packaged food products. Hydrocarbons, ketones, and aromatic compounds are able to migrate into a packed food product and affect its quality [124]. The kinetics of degradation showed that some compounds remain trapped in the polymer and can be used as irradiation detectors. Irradiated foods should be properly handled and stored after treatment to avoid deterioration, spoilage, and loss of nutritive value. Thus, handling, storage conditions, and packaging are also important postirradiation considerations [2].

47.4.2 Legal Aspects and Safety Issues

A joint FAO/IAEA/WHO Expert Committee on Food Irradiation (IJECFI) concluded that irradiation of food up

TABLE 47.9
Packaging Materials Approved by FDA for Use during Irradiation of Food Packaged Materials

Type of Material	Maximum Dose (kGy)
Paper (Kraft)	0.5
Paper (glassine)	10
Paperboard (wax-coated)	10
Cellophane (coated)	10
Polyolefin film	10
Polystyrene film	10
Rubber HCl film	10
Vinylidene chloride–vinyl chloride (copolymer film)	10
Nylon-6	10
Vegetable parchment	60
Polyethylene terephthalate film	60
Nylone-11	60
Vinyl chloride–vinyl acetate copolymer film	60
Acrylonitrile copolymers	60

Source: Willemoti et al. [144].

to an overall average dose of 10 kGy causes no toxicological hazards and introduces no special nutritional or microbiological problems [127]. Later, other organizations, such as Health Canada, FDA, Codex Alimentarius Commission, and the European Commission's Scientific Committee on Food also supported this limit [128]. In 1993, the American Medical Association's Council on Scientific Affairs endorsed food irradiation as a safe and effective tool to increase food safety and reduce the incidence of foodborne illness, a view expressed earlier by the U.S. Department of Agriculture [3].

Irradiation of food and agricultural products is currently allowed by about 40 countries and approximately 60 commercial irradiation facilities are operating in the United States [75]. The most common irradiated food products for commercial use are spices and dry vegetable seasonings [3]. Loaharanu and Murrell [3] mentioned that the recent ban by the European Union on the use of ethylene oxide for food could increase the quantity of spices and vegetable seasonings processed by irradiation in the near future. The fumigant ethylene oxide is reported to be carcinogenic and methyl bromide could be harmful to the ozone layer [129].

The safety issues of irradiated foods can be grouped as [9] (i) residual radioactivity, (ii) free radicals and radiolytic products, (iii) carcinogenic and mutagenic properties, (iv) nutrient quality, (v) polyploidy, (vi) toxicity, (vii) microbiological safety, and (viii) operator safety during processing. A complete review of the above aspects of safety is provided by Smith and Pillai [128]. The safety for human consumption of irradiated products has been frequently questioned [13]. The vast majority of toxicological studies and feeding trials have showed no evidence of toxic effects. However, some studies claimed to find evidence of polyploidy in irradiated wheat fed to children [5]. There is already a wide experience in the design, building, and operation of irradiation plants. Therefore, the plants must be controlled and inspected by authorities in order to ensure the national and international radiological safety standards, for example, the health and safety of workers, and environmental pollution from radioisotopes [130].

Relatively small doses of irradiation can reduce the number of pathogenic organisms to very low levels. This may cause a rise in the growth of secondary microflora, such as *Moraxella*, lactic acid bacteria, and yeasts. Thus, Mitchell [4] expressed concerns about in the absence of competing spoilage microorganisms postirradiation, toxin production by surviving pathogens may occur more quickly making the food unsafe to eat before it is visibly spoiled [131]. Irradiation is not an excuse for poor hygiene and is not to be used for reducing unacceptably high levels of microbial contamination [5]. Smith and Pillai [128] identified two major concerns expressed by anti-irradiation groups: misuse to avoid plant sanitation and environmental concerns. The volume of irradiated foods is increasing and the future of irradiated foods is as bright as ever [132].

47.4.3 Consumer Attitudes

The application and cost-effectiveness of irradiation as a method to control foodborne pathogens will depend on consumer attitudes, regulatory actions, economics, and logistics associated with different situations. Moy [9] mentioned four reasons for the slow commercialization of food irradiation: antinuclear activists, the industry's hesitation, time-consuming approval process, and insufficient consumer education. Griffith [133] mentioned that the major factors that will determine the future of food irradiation are the development of a simple and reliable detection method, the harmonization of legislation, the commitment of the food industry, and consumer attitudes. Similar to genetic engineering techniques in food production, it is essential that consumer education and consultation with consumers are an integral part of future developments. Loaharanu [14] reviewed the results of consumer attitude surveys in different countries. He concluded that in advanced countries, consumers at large are still not knowledgeable about food irradiation. They need accurate information about safety, benefits, and limitations of food irradiation.

Hashim et al. [134] also reviewed consumer attitudes toward irradiated poultry and recommended ways to increase the acceptance of irradiated foods: (i) educational programs to increase consumer understanding about irradiation;, (ii) propagate information about safety of irradiation through labels and/or posters; (iii) television shows, children interactions, and/or pamphlets or brochures; and (iv) in-store sampling of cooked irradiated poultry. Recently, the importance of consumer education is also identified [135–137]. Pohlman et al. [138] also showed that audiovisual presentation affects consumers' knowledge and attitude toward food irradiation. Kilcast [5] mentioned three methods have been developed that can currently be used for detection purposes. These are electron spin resonance, thermoluminescence, and detection of lipid breakdown volatiles. However, all these methods require specialist expertise.

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48 Pulsed Electric Fields in Food Preservation

Humberto Vega-Mercado, M. Marcela Gongora-Nieto, Gustavo V. Barbosa-Canovas, and Barry G. Swanson

CONTENTS

48.1 INTRODUCTION

There are many different forms in which to apply electric energy for food pasteurization. These include ohmic heating [1–3], microwave heating [4–6], low electric field stimulation [7, 8], high-voltage arc discharge [9–12], and high-intensity pulsed electric field (PEF) application [13–15]. Ohmic heating is one of the earliest forms of electricity applied to food pasteurization [1]. This method relies on the heat generated in food products when an electric current is passed through them. Getchell [2] described the ohmic heating method in milk pasteurization. A 220 V, 15 kW alternating current supply was applied to milk through carbon electrodes in an electrical heating chamber. The milk was heated to and held at 70°C for about 15 seconds. It has been reported that ohmic heating is suitable for viscous products and foods containing particles, and this method is considered to be a promising technique for the aseptic processing of foods [3].

Microwave heating has been extensively applied in everyday households and the food industry [4]. Many food materials possess very low values of static conductivity. However, when they are subjected to microwave fields, they exhibit very high values of alternating field conductivity and consume considerable energy [5]. The heat generated by microwaves is used for heating processes. Studies on microbial inactivation using microwave energy have concluded that microbial death is caused solely by thermal mechanisms [6].

Low electric field stimulation has been explored as a method of bacterial control of meat. In electrical stimulation of meat, an electric field of 5–10 V/cm is applied as alternate current (ac) pulses to the sample through electrodes fixed at opposite ends of the long axis of the muscle [7]. Recently, a very low field (0.4 V/cm) was applied in a 6 liter treatment medium in search of an easy, safe, and practical method to eliminate bacteria for food-processing purposes. Several species of bacteria in saline solution were inactivated [8]. Salt solutions and their concentrations play a very important role in this method.

Inactivation of microorganisms and enzymes contained in food products by electric discharge began in the 1920s with the Electropure process for milk [16], which consisted of passing an electric current through carbon electrodes and heating milk to 70°C to inactivate *Mycobacterium tuberculosis* and

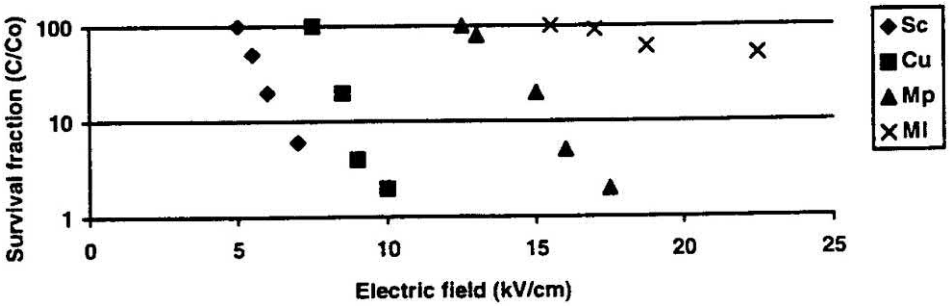

FIGURE 48.1 Relationship between survival fraction and electric field strength (1020 μs pulses) (Sc: *Saccharomyces cerevisiae*; Cu: *Candida utilis*; Mp: Motile pseudomonad; MI: *Micrococcus lysodeikticus*). (Adapted from Hamilton and Sale [27].)

Escherichia coli. Beattie and Lewis [17] demonstrated the lethal effect of electrical discharges on microorganisms when the applied voltage used to treat food was increased 3000–4000 volts. The electrohydraulic treatment was introduced in the 1950s to inactivate microorganisms suspended in liquid foods. The inactivation of microorganisms was attributed to a shock wave generated by an electric arc that prompted the formation of highly reactive free radicals from chemical species in food [14]. Gilliland and Speck [18] applied pulsed electric discharges at different energy levels for the inactivation of *E. coli*, *Streptococcus faecaiis*, *Bacillus subtilis*, *Streptococcus cremoris*, and *Micrococcus radiodurans* suspended in sterile distilled water as well as for trypsin and a protease from *B. subtilis.*

Sale and Hamilton [19] demonstrated the nonthermal lethal effect of homogeneous electric fields on bacteria such as *E. coli*, *Staphylococcus aureus, Micrococcus iysodeikticus*, *Sarcina lutea*, *B. subtilis*, *Bacillus cereus*, *Bacillus megaterium*, and *Clostridium welchii*, and yeasts such as *Saccharomyces cerevisiae* and *Candida utilis.* In general, an increase in the electric field intensity and number of pulses was found to lead to an increase in the inactivation of microorganisms (Figure 48.1 and Table 48.1). Other factors that influence microbial inactivation by pulsed electric fields are the treatment temperature, pH, ionic strength, and conductivity of the medium containing the microorganisms [9, 20–26].

The formation of pores on cell membranes by high-intensity pulsed electric fields (HIPEFs) is not entirely understood.

TABLE 48.1
Activity of *Staphylococcus aureus* after Pulsed Electric Field Treatment

Electric Field (kV/cm)	Survivors (%)	Protoplasts Not Lysed
0.00	100	100
9.25	100	100
14.25	35	43
19.50	0.9	16
24.00	0.3	3
27.50	0.6	2

Source: Adapted from Hamilton and Sale [27].

Zimmermann et al. [28], applying the dielectric rupture theory, concluded that membrane rupture is caused by an induced transmembrane potential approximately 1 V larger than the natural potential of the cell membrane. The reversible or irreversible rupture (or electroporation) of a cell membrane depends on factors such as intensity of the electric field, number of pulses, and duration of the pulses [29–32]. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field; permeation then causes swelling and the eventual rupture of the cell membrane (Figure 48.2).

In September 1996, the U.S. Food and Drug Administration (FDA), based in Washington, D.C., released a "letter of no objection" for the use of pulsed electric fields to treat liquid eggs. To meet FDA requirements [33] in filing a new and a novel process, it is necessary to (i) establish an active and continuous dialog with the FDA during process development, (ii) meet with the FDA to describe the process, (iii) invite the FDA to a site visit (pilot and production facility), and (iv) draft and provide the FDA with an outline of the proposed filing.

The objective of the FDA is to conduct a scientific evaluation of the process to determine if the aseptically produced product poses a potential public health hazard and if all of the critical factors necessary to render the product commercially sterile are monitored and controlled. The filing information of the new process must contain

1. Equipment design: a description of the system, control mechanisms used and fail safe procedures
2. Product specifications: a full description of the product, including physical/chemical aspects, critical factors, and influence of processing on the critical factors
3. Process design: a complete description of the critical/processing conditions used in the manufacture of the product
4. Validation: a physical demonstration of the accuracy, reliability, and safety of the process

In the area of pulsed electric fields, there are many possible project-development designs related to (i) unknown destruction kinetics of microbial pathogens (e.g., *Clostridium botulinum*), (ii) identification of proper indicator organisms, (iii)

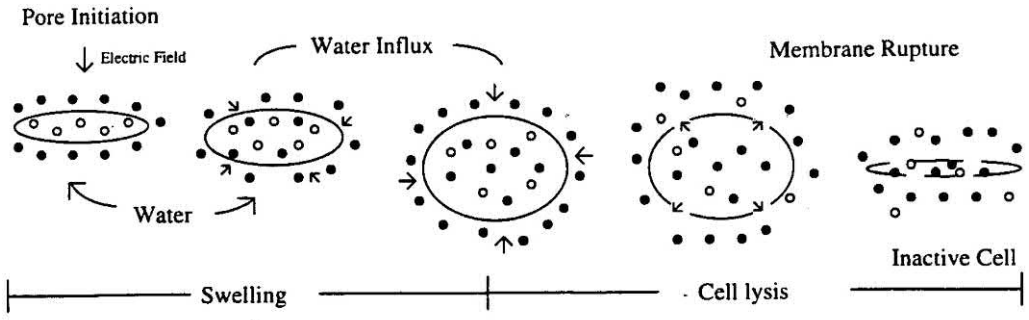

FIGURE 48.2 Mechanism of cell inactivation. (Adapted from Tsong [31].)

uniformly delivered treatment, (iv) impact of processing conditions (e.g., temperature, pH, moisture, and lipid content), (v) identification/monitoring of critical factors (e.g., surface, intensity), and (f) food additives.

48.2 ENGINEERING ASPECTS OF PULSED ELECTRIC FIELDS

The concept of pulsed power is simple: electric energy at low power levels is collected over an extended period and stored in a capacitor. That same energy can then be discharged almost instantaneously at very high levels of power. The generation of pulsed electric fields requires two major devices: a pulsed power supply and a treatment chamber, which converts the pulsed voltage into pulsed electric fields.

48.2.1 Bench-Top Unit

A commercial electroporator (i.e., GeneZapper, IBI-Kodak Company, Rochester, New York) may be used as a bench-top pulsed power supply. This unit provides a maximum of 2.5 kV pulses. The instrument consists of a capacitor (7 μF), charge and discharge switches, and a wave controller. The wave controller may be connected to the electroporator to improve the discharge pattern. Treatment cuvettes with a 0.1 cm electrode gap and l00 μl volume may be used for PEF treatments, which give a maximum intensity of approximately 25 kV/cm. Appropriate voltage and current monitors should be attached to the GeneZapper to measure the pulsed electric field treatments. Figure 48.3 illustrates the major components of the GeneZapper. This bench-top unit provides a convenient method for determining the inactivation kinetics for selected microorganisms.

48.2.2 Lab Scale Pulser

Exponential decay electric pulses can be generated by discharging a capacitor into a chamber containing the food (Figures 48.4–48.6). Current designs for power supplies are able to provide up to 40 kV. Capacitors of 5 μF are used to store the electric energy that is discharged across metal electrodes, creating the electric field used to inactivate microorganisms and enzymes. A mercury ignitron spark gap may be used as the discharge switch. This type of unit may be employed for inactivation studies in a continuous mode. Pulsed voltage across the treatment chamber may be monitored by a resistance voltage divider. Electric current may be monitored by a Rogowski coil connected to a passive integrator. Both voltage and current waveforms may be monitored using a digital oscilloscope.

48.2.3 Treatment Chambers

A static PEF treatment chamber consists of two electrodes held in position by insulating materials that also form an enclosure containing food materials. Uniform electric fields can be achieved by parallel plate electrodes with a gap sufficiently smaller than the electrode surface dimension. Disk-shaped, round-edged electrodes can minimize electric field enhancement and reduce the possibility of dielectric breakdown of

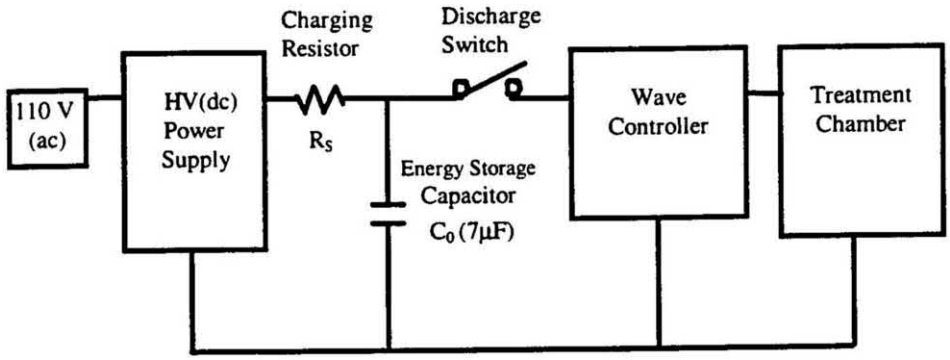

FIGURE 48.3 Major components of commercial electroporator GeneZapper.

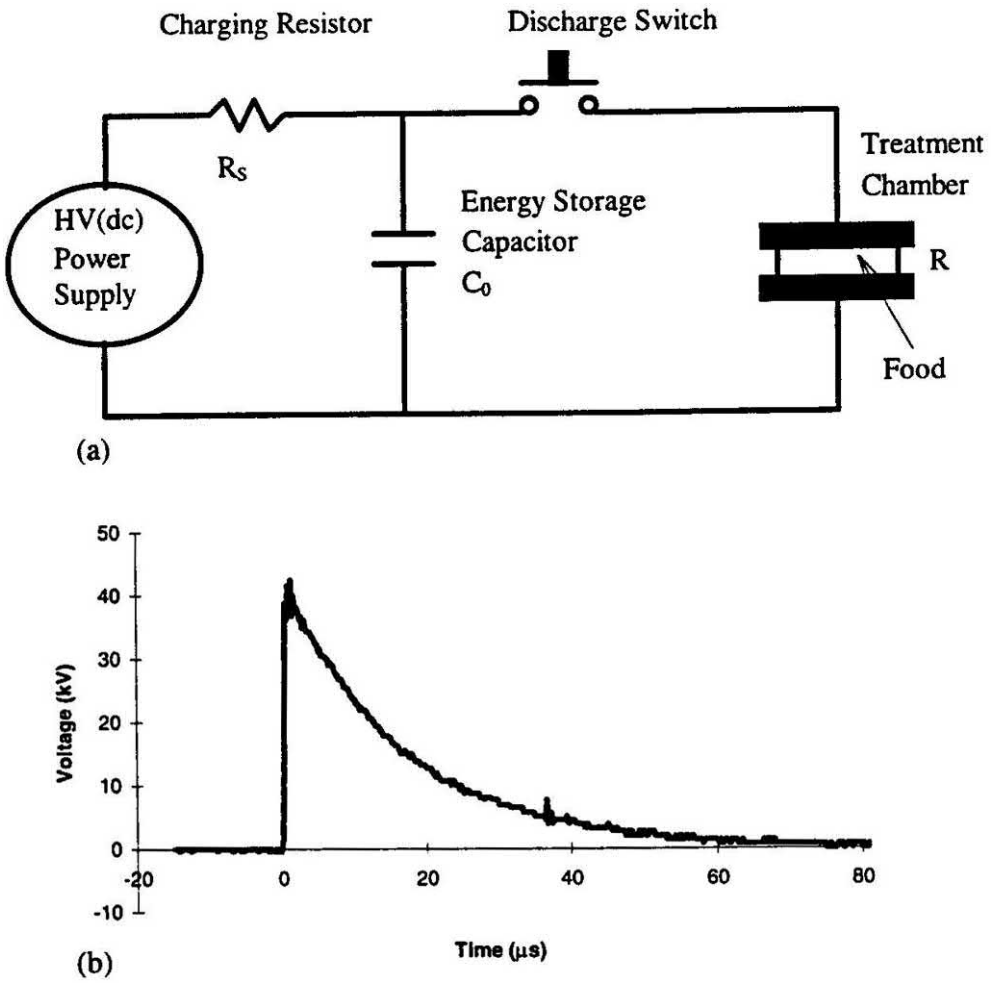

FIGURE 48.4 (a) A simplified circuit for producing exponential decay pulses and (b) a voltage trace across the treatment chamber.

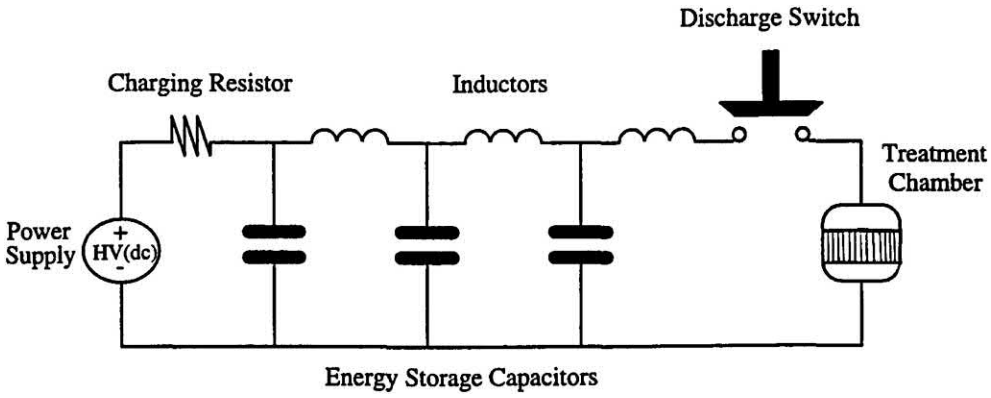

FIGURE 48.5 Typical pulser configuration for high-intensity pulser electric fields.

fluid foods. A continuous flow-through treatment chamber (Figure 48.7) was developed at Washington State University (WSU) to test the flow-through concept using low flow rates. The chamber consisted of two electrodes, a spacer, and two lids. Each electrode was made of stainless steel, whereas the spacer and lids were made of polysulfone. A flow channel was provided between the two electrodes to eliminate dead corners as well as to ensure uniform treatment.

The operating conditions for the parallel plate continuous chamber were as follows: chamber volume, 20 or 8 cm^3 electrode gap 0.95 or 0.51 cm; PEF, intensity 35 or 70 kV/cm; pulse width, 2–15 μs; pulse rate, 1 Hz; and food flow rate, 1200 or 600 cm^3/min. Cooling of the chamber was accomplished by circulating water at a selected temperature through jackets built into the two stainless steel electrodes. It should also be pointed out that a completely sealed treatment chamber

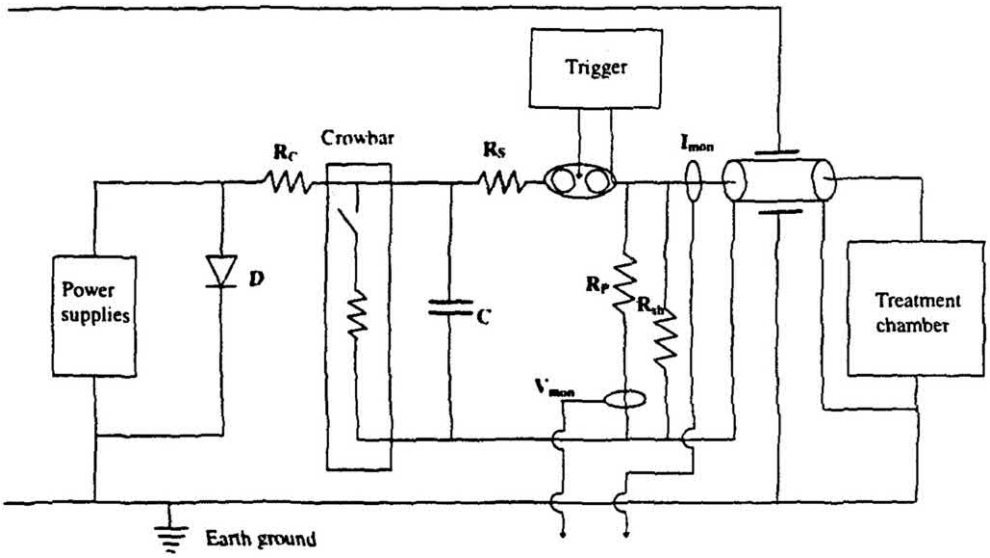

FIGURE 48.6 Current setup of the PEF facility at Washington State University. The pulser has a 16 kJ/s charging power supply, 40 kV peak charging voltage, and 10 Hz pulse repetition rate. C: storage capacitor; D: power supply protection diode; R_c: charging resistor; R_s: series resistor; R_{sh}: shunt resistor; R_p: voltage-measuring resistor; I_{mon}: current monitor; V_{mon}: voltage monitor.

is dangerous. When the test fluid experiences a spark, high pressure develops rapidly and the chamber may break apart. A pressure-released device must be included in the treatment chamber design to ensure the safety of the operation.

A coaxial treatment chamber (Figure 48.8) with a uniform field distribution along the fluid path was designed at WSU. The fluid is fed into the chamber through the bottom region and the treated product exits at the top of the chamber. The protruded surface, located at the outer grounded electrode, enhances and makes uniform the electric field within the treatment region while it reduces the field intensity in other regions of the fluid path. Cooling fluid is circulated to control the temperature between the inner high-voltage electrode and the outer grounded electrode. The gap in the coaxial electrode or the liquid food thickness along the direction of the electric field can be selected by changing the diameter of the inner electrode.

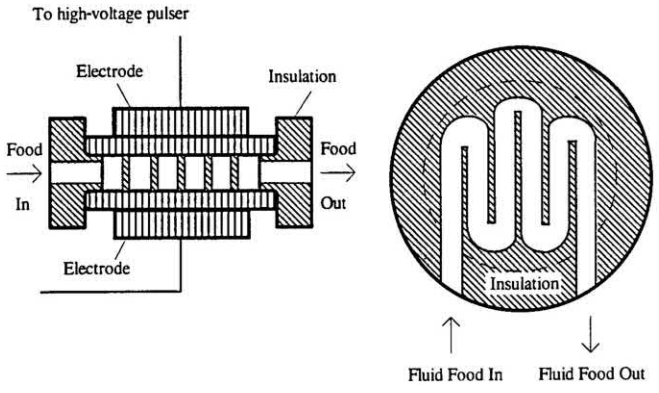

FIGURE 48.7 Schematic drawing of a flow-through treatment chamber. Fluid inside the chamber is baffled to avoid dead spots.

48.2.4 Pulsed Electric Field Process Design

48.2.4.1 HACCP Principles and PEF Technology

The PEF process is summarized in Figure 48.9. The key operations are the receiving of raw materials, PEF treatment, aseptic packaging operation, and finished product storage and distribution. The following analysis [34] is based upon the seven principles of hazard analysis and critical control points (HACCP).

48.2.4.1.1 Hazard Assessment

Microbial hazards are the main concern throughout the PEF operation. Raw materials contain spoilage microbes and

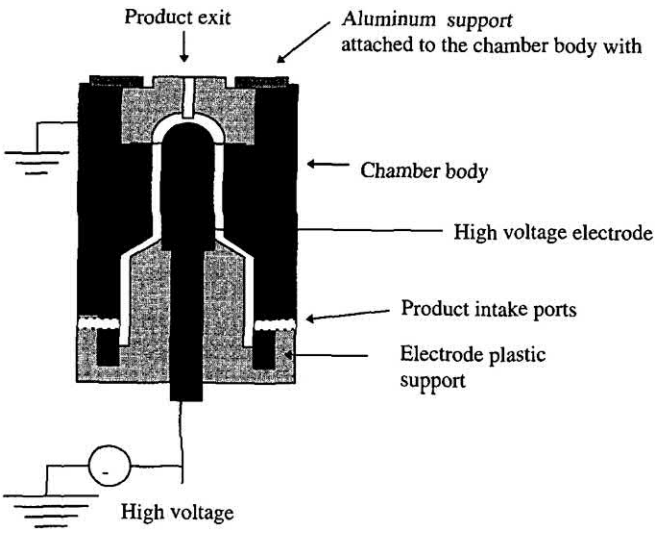

FIGURE 48.8 Schematic of the REF continuous treatment chamber.

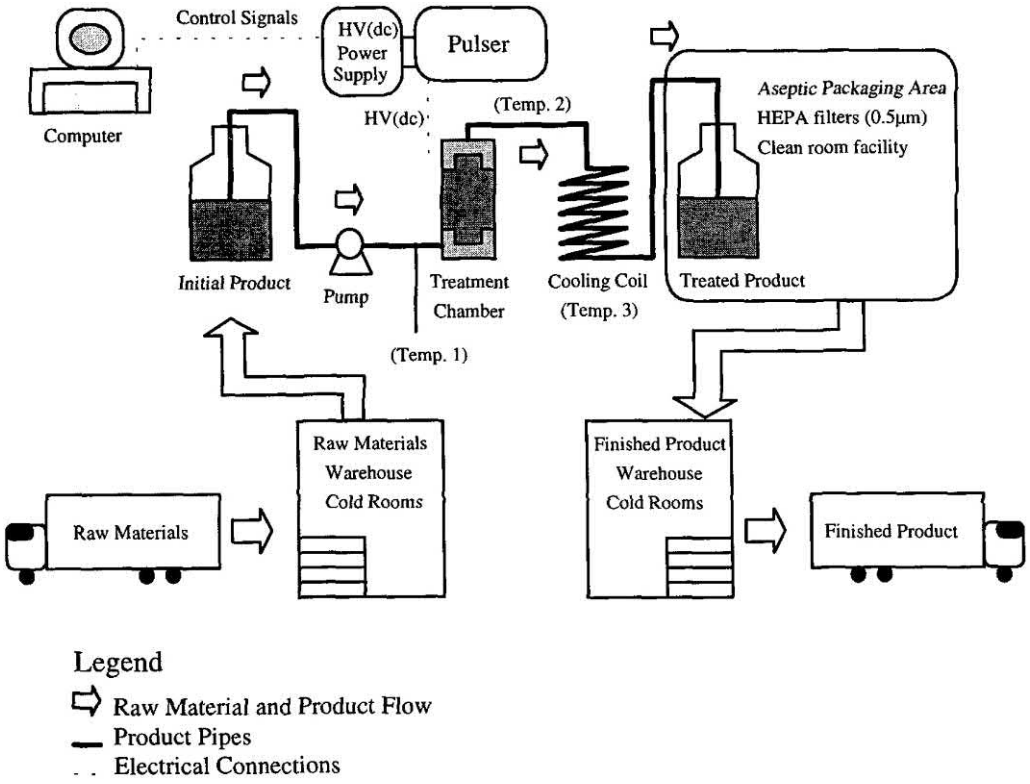

FIGURE 48.9 Pulsed electric field unit operations layout.

pathogens that may spoil the ingredient or raw material, or may be harmful to the consumer. Storage facilities for raw materials may increase the risk of microbial contamination from soil and water deposits. The cleanliness of processing equipment plays a key role in preventing microbial contamination, thus the multiple assembly parts must always be properly sanitized. Inappropriate aseptic packaging operations and storage conditions may result in spoilage of the product.

Chemical hazards to consider are the presence of antibiotic and pesticide residuals on raw materials, electrically induced chemical reactions, and excessive detergent/sanitizer residues from processing and packaging equipment. Physical hazards include foreign matter in raw materials (e.g., stones, rubber, plastic, metal, and eggshells), metal particles from the treatment chamber after a spark, and plastic or rubber pieces from seals.

The final risk classification may be defined in terms of the product (milk, apple juice, eggs, soups, etc.). Six microbiological hazard characteristics, as well as chemical and physical hazard characteristics, are defined by the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) and will be used to classify PEF products. In general, the final hazard classification should occur between risk categories IV and VI as defined by the NACMCF.

48.2.4.1.2 Critical Control Points: Determination, Limits, Procedures, and Corrective Actions

The following critical control points (CCPs) should be selected to ensure the safety of PEF products: receiving and storage section, PEF treatment section, and aseptic packaging section. The main factors considered and monitored for each CCP are handling and processing time, temperature of material, and cleanliness of equipment and utensils. The treatment conditions (electric field intensity, pulsing rate, input voltage, input current, and chamber temperature) should be monitored and recorded on a continuous basis. Uniform PEF treatment requires the design and construction of a pulser that accomplishes variable pulsing rates, charging rates, voltage settings, pulse widths, and pulse shapes. Pulser components such as power source, computerized controls, triggering mechanism, overloads, dummy loads, and treatment chamber should comply with defined specifications and characteristics such as maximum operating temperature, maximum voltage and current outputs, and reliability (mean time between failures, yields, etc.). The reliability of the pulser may be measured in terms of number of pulses with correct energy level per unit of time as well as total pulses per unit of time. Monitoring devices may include oscilloscopes for voltage and current measures, and pulse counters.

Standard operating procedures (SOPs) should be in place to define aspects such as reception, storage, and preparation of raw materials to ensure proper handling and to reduce the risk of contamination. The pulsing and packaging units must have procedures to specify the assembly and disassembly of the machinery. Cleaning specifications such as frequency and type of detergents and sanitizers to use should be established to prevent contamination between products. The operational parameters for PEF treatments must be specified for each food product based upon its microbial risk, initial microbial counts,

physical and chemical characteristics (e.g., pH, ionic strength, composition), and the maximum time to complete the processing of each food (i.e., time from initial discharge of raw materials to the end of the packaging operation). Alternative procedures must define the corrective actions associated with deviations from process specifications or CCP limits. Quality assurance procedures must be developed for the approval or rejection of PEF-treated products based on the CCP limits and corrective actions.

48.2.4.1.3 Record Keeping

Record keeping is a key aspect not only in a PEF operation but in any successful manufacturing operation. The status of raw materials, process and packaging sequence, and storage and shipping procedures must be reflected in the batch or lot documents. Proper design of the documents is an important and difficult task because the documents must provide enough space for critical measurements without confusing the operator.

48.2.4.2 Hazard and Operability Study (HAZOP) Principles and PEF Technology

The main concern of individuals working in a PEF facility is the voltage intensity, which reaches the kilovolt range. A typical pulser configuration is presented in Figure 48.10. A high-voltage power supply is selected to charge the capacitor (eventually more than one) and a discharge switch releases the stored electric energy from the capacitor through the product in the form of an electric field. The power supply, capacitor, and treatment chamber must be confined in a restricted access area with interlocked gates. The gates will turn off the pulser if they are opened while the power supply is on. Emergency switches must be accessible in case of a process failure. Also, discharging bars must be provided to discharge the elements in the circuit before maintenance or inspection of the unit occurs. To prevent the leakage of high voltage through any fluid (food or refrigerant) in contact with the treatment chamber, all connections to the chamber will be isolated and the pipes carrying materials to or from the chamber connected to the ground.

Electrical and mechanical devices such as pumps, computers, and packaging machines must be protected using safeguards. Proper warning signs must be in place regarding the safety hazards (high voltage, high-intensity electric field) in the processing area. The information related to the operation and maintenance procedures must be contained in standard operating procedures (SOPs). The personnel involved in the PEF operation must be trained and instructed in these SOPs.

The selection of appropriate detergents and sanitizers must comply with the FDA and U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) regulations, or regulations of equivalent organizations in other countries. Proper protection devices such as face masks or goggles, aprons, boots, and gloves must be used by employees while applying and removing the cleaning solutions. A complete procedure must be in place to define what kind, when, where, and how to use the cleaning and sanitizing solutions. Proper record keeping is required to avoid contamination of the products with detergent or sanitizer solutions. A complete layout of the facility including details about location of utilities, location of equipment, and emergency exits must be available. Changes in the configuration of the facility must be reflected in the layout.

48.2.5 Currently Used PEF Technology

PurePulse Technologies Co., a subsidiary of Maxwell Laboratories in San Diego, California, owns three U.S. patents to preserve fluid foods such as dairy products, fruit juices, and fluid eggs by treatment with high-intensity electric

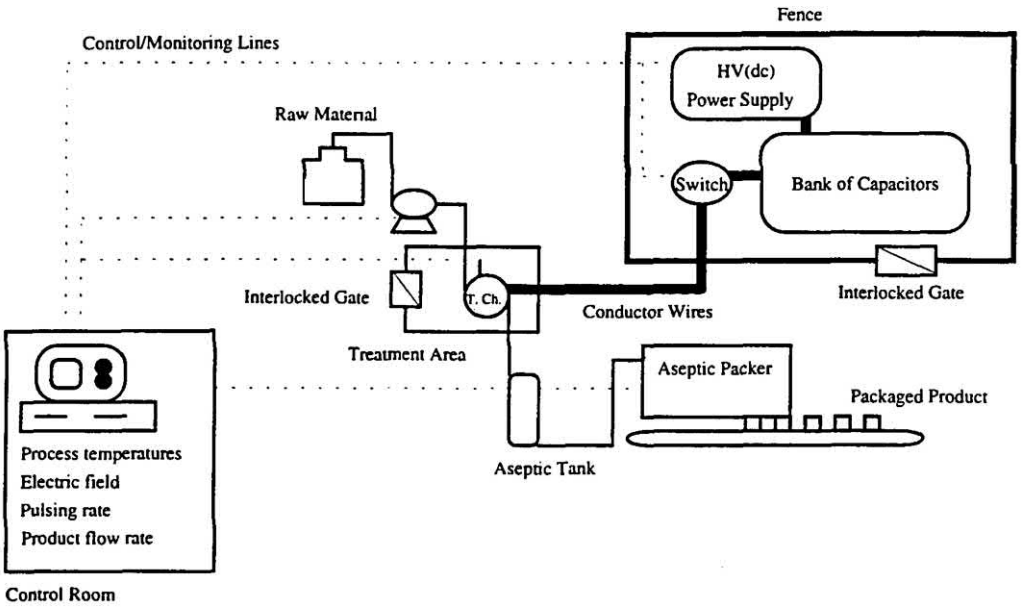

FIGURE 48.10 Schematic diagram of a PEF equipment configuration.

discharges from about 5 to 100 kV/cm with flat-topped exponentially decaying pulse shapes. Pulse duration is controlled to prevent electrical breakdown of the food product; the typical duration is between 1 and 100 μs with repetition rates between 0.1 and 100 Hz [11, 35]. The patents describe both a batch and continuous processing system and recommend that HIPEF treatments be applied to preheated liquid foods, which enhance microbial inactivation and shelf-life stability.

Dunn and Pearlman [11] reported more than five logarithmic cycles of microbial count reduction (5D reduction) of naturally occurring microorganisms in orange juice after 35 pulses of 100 μs at a voltage intensity of 33.6–35.7 kV/cm and a process temperature of 42–65°C. The shelf life of orange juice was increased from 3 days to one week with no significant change in odor or taste. A 3D reduction of *E. coli* (ATCC-10536) inoculated in homogenized and pasteurized milk exposed to 23 pulses of 100 μs at 28.6–42.8 kV/cm was also reported. When a similar test run was carried out using milk seeded with *Salmonella dublin* prior to treatment with 36.7 kV/cm and 40 pulses of 100 μs at 63°C, no *Salmonella* and only 20 cfu/ml of milk bacteria was found. These results may suggest that deactivation from the PEF treatment process is selective and that *S. dublin* are preferentially deactivated over the milk bacteria. Yogurt inoculated with *Streptococcus thermophilus*, *Lactobacillus bulgaris*, and *Saccharomyces cerevisiae* was treated with 20–100 μs pulses at 23–38 kV/cm at a process temperature of 63°C, resulting in a 2D reduction of the lactic acid bacteria and *S. cerevisiae* [11].

The *ELSTERIL* process, developed by Krupp Maschinentechnik GmbH (Hamburg, Germany) in the late 1980s and early 1990s, is used for the sterilization and pasteurization of liquid and electrically conductive media [13, 36, 37]. Krupp Maschinentechnik GmbH, in association with the University of Hamburg, reported microbial inactivation when PEF was applied to fluid foods such as orange juice and milk [37]. A microbial inactivation exceeding 4D has been found for *Lactobacillus brevis* inoculated in milk and treated with 20 pulses of 20 ps at 20 kV/cm, *S. cerevisiae* inoculated in orange juice and treated with 5 pulses of 20 μs at 4.7 kV/cm, and *E. coli* inoculated in sodium alginate and treated with 5 pulses of 20 μs at 14 kV/cm [36, 37]. However, no inactivation of the endospores of *B. cereus* or the ascospores of *Bacillus nivea* was reported [37]. A substantial reduction in ascorbic acid and lipase activity was observed in milk treated with the ESTERIL process [37]. The taste of milk and orange juice did not significantly change after the electric field treatments [37].

The disruption of cell membranes to release fat from animal cells was conducted using a process called *ELCRACK* (Krupp Maschinentechnik GmbH, Hamburg, Germany). The ELCRACK process consists of the exposure of a slurry of comminuted fish or slaughterhouse offal to high-intensity electric pulses that break down cells, leading to increased fat recovery during the separation step after it is pumped through one or more treatment chambers [36]. Washington State University has a patent for the design and development of a static PEF chamber and has filed another for the design and development of a continuous PEF chamber intended for processing liquid foods with PEF treatments [38–41].

48.3 APPLICATIONS OF PEF IN FOOD PROCESSING

The application of PEF as a food-processing tool is gaining popularity since it represents a nonthermal alternative to conventional pasteurization and sterilization methods. The PEF approach, which does not involve the use of added preservatives, is expected to be more appealing to consumers who are skeptical about the use of chemicals in foods. Furthermore, the PEF treatment, being a nonthermal process, may also have no significant detrimental effect on heat-labile components present in foods such as vitamins. The major disadvantage of PEF operation is the initial investment. A pilot plant-size pulser may cost around $250,000. Other units for industrial use are available at prices that range from $450,000 to $2,000,000.

48.3.1 Inactivation of Microorganisms

Raw and reconstituted apple juice, peach juice, skim milk, beaten eggs, and pea soup exposed to PEFs of 25–45 kV/cm were treated using the chamber designed at Washington State University. *E. coli* inoculated in skim milk and exposed to 60 pulses of 2 μs width at 45 kV/cm and 35°C was reduced by 2D [25]. A reduction of 6D was observed in liquid egg inoculated with *E. coli* and treated with an electric field of 25.8 kV/cm and 100 pulses of 4 μs at 37°C [42]. *E. coli* and *B. subtilis* inoculated in pea soup and exposed to PEFs of 25–33 kV/cm (10–30 pulses of 2 μs) provided a limited inactivation (<1.5D) when the process temperature of pea soup was below 53°C, while microbial inactivation was 4.4D with process temperatures between 53°C and 55°C [26].

48.3.1.1 Simulated Milk Ultrafiltrate (SMUF)

The inactivation of *E. coli* varied as a function of the electric field intensity, number of pulses, and pH. Low field intensity (20 kV/cm) resulted in insignificant inactivation of microorganisms independent of temperature and pH ($p > 0.05$). Meanwhile, inactivation of *E. coli* increased with an increase in the number of pulses and an increase in the electric field from 40 to 55 kV/cm. The inactivation was more significant at pH 5.69 than at pH 6.82 ($p < 0.05$) (Figures 48.11A and 48.11B). The temperature effect (10°C or 15°C) on the inactivation for these experiments was not statistically significant ($p > 0.05$). Table 48.2 summarizes the inactivation results after eight pulses for each of the experimental conditions.

The role of pH in the survival of microorganisms is related to the ability of the organisms to maintain the cytoplasm pH near neutrality [43]. Membrane permeability increases due to the formation of pores in the cell wall during PEF treatment [31] and the rate of transport of hydrogen ions may also increase due to the osmotic imbalance around the cell. Thus, a reduction in cytoplasm pH may be observed because a higher number of hydrogen ions are available than at a neutral pH. The change in pH within the cell may induce chemical

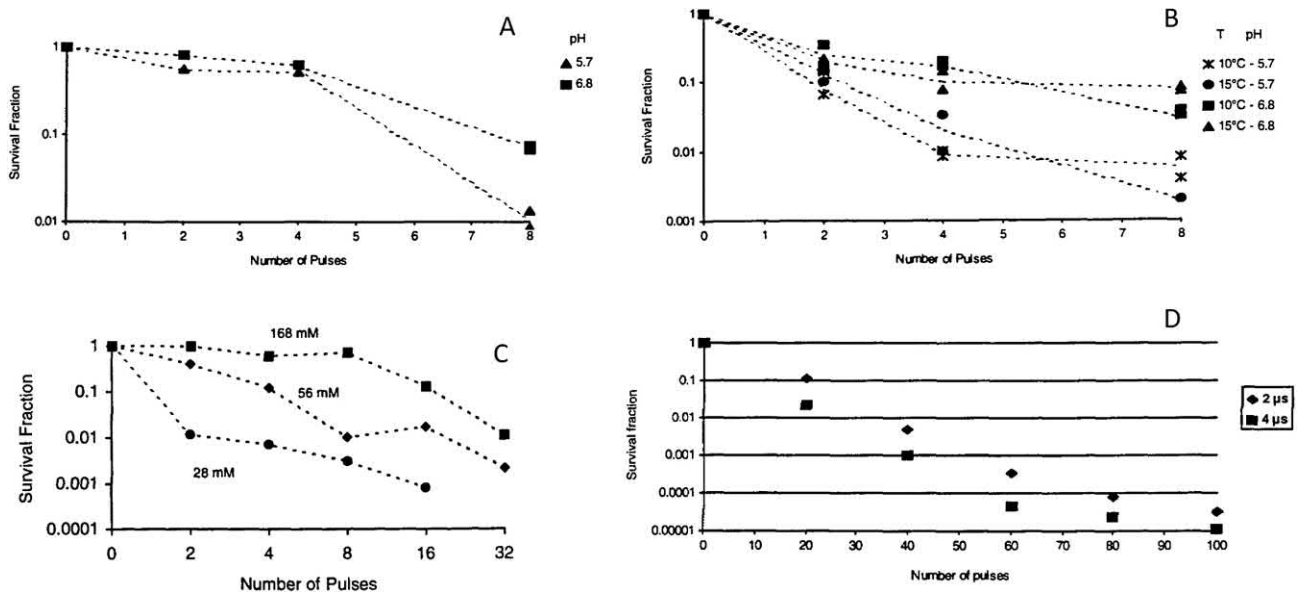

FIGURE 48.11 (A) Inactivation of *E. coli* suspended in SMUF, using 40 kV/cm at 10°C, two samples per each experimental condition. (From Verga-Mercado et al. [26].) (B) Inactivation of *E. coli* suspended in SMUF, using 55 kV/cm, two samples per each experimental condition. (From Verga-Mercado et al. [26].) (C) Effect of ionic strength on the inactivation of *E. coli* suspended in SMUF, at 40 kV/cm and 10°C, two samples per each experimental condition. (From Verga-Mercado et al. [26].) (D) *E. coli* in liquid egg after PEF treatment at 26 kV/cm and 37°C in a continuous recirculation system. (From Martin et al. [42].)

modifications in fundamental compounds such as DNA or ATP, as discussed by Wiggins [44] and Dolowy [45]. Also, oxidation and reduction reactions such as those proposed by Gilliland and Speck [18] may occur within the cell structure induced by the PEF treatment.

The ionic strength of the solution also plays an important role in the inactivation of *E. coli*. An increase in the ionic strength increases the electron mobility through the solution, resulting in a decrease in the inactivation rate. The reduced inactivation rate in high-ionic strength solutions can be explained by the stability of the cell membrane when exposed to a medium with several ions [31]. The effect of ionic strength can be observed in Figure 48.11C, where a difference of 2.5 log cycles was obtained between the 0.168 and 0.028 M solutions.

The growth stage of *E. coli* affected the effectiveness of PEF treatments (36 kV/cm at 7°C, two and four pulses). Cells in the logarithmic phase were most sensitive to the electric field treatments compared to cells in the stationary and lag phase (Figure 48.12) as reported by Pothakamury et al. [46]. Figures 48.13A and 48.13B present the effect of temperature on the log-cycle reduction of *E. coli* using exponentially decaying pulses and square wave pulses of 35 kV/cm. The rate of inactivation increases with an increase in temperature. Coster and Zimmermann [47] suggested synergistic effects of high-intensity electric fields with moderate temperatures. The rate of inactivation increased when square wave pulses were used compared to exponentially decaying pulses. Similar results were reported for *S. aureus* when exposed to PEF at 9 and 16 kV/cm and *L. delbrueckii* and *B. subtilis* when exposed to 9, 12, and 16 kV/cm. Figures 48.13C, 48.13D, and 48.14A present the reported results by Pothakamury et al. for *S. aureus, L. delbrueckii*, and *B. subtilis* suspended in SMUF.

48.3.1.2 Pea Soup

PEF inactivation of *E. coli* and *B. subtilis* suspended in pea soup depends on the electric field intensity, number of pulses, pulsing rate, and flow rate [49] (Table 48.3). The maximum bulk temperature of the peak soup achieved during the PEF treatment was 55°C and is a function of both flow rate and pulsing rate. PEF treatments with a bulk temperature below 53°C resulted in limited microbial inactivation (<1.64D). Microbial inactivation dependence on process temperature may be explained by changes in the sensitivity of the microorganisms to PEF when the temperature exceeds 53°C. Thermal inactivation of microorganisms was avoided by cooling treated pea

TABLE 48.2
Effect of Processing Parameters on the Inactivation of *E. coli* Suspended in SMUF after Eight Pulses

Description	pH	Number of Log-Cycle Reduction	
		10°C	15°C
20 kV/cm	5.7	0.00[a]	0.20[a]
	6.8	0.00[a]	0.06[a]
	5.7	1.95[b]	1.85[b]
	6.8	1.16[c]	1.00[c]
	5.7	2.22[d]	2.56[d]
	6.8	1.45[e]	1.1[c]

Source: Verga-Mercado et al. [26]

Note: Log-cycle reduction data with similar superscripts are not significantly different at $\alpha = 0.05$, two samples per each experimental condition.

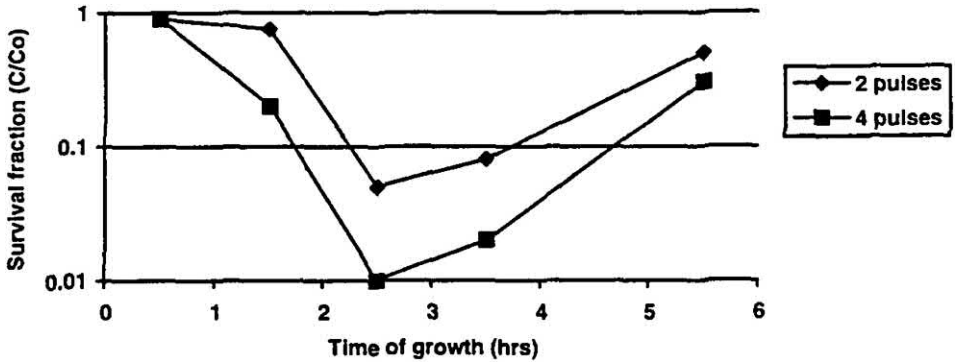

FIGURE 48.12 Effect of growth stage on the pulsed electric field inactivation of *E. coli* suspended in SMUF. (From Pothakamury et al. [46].)

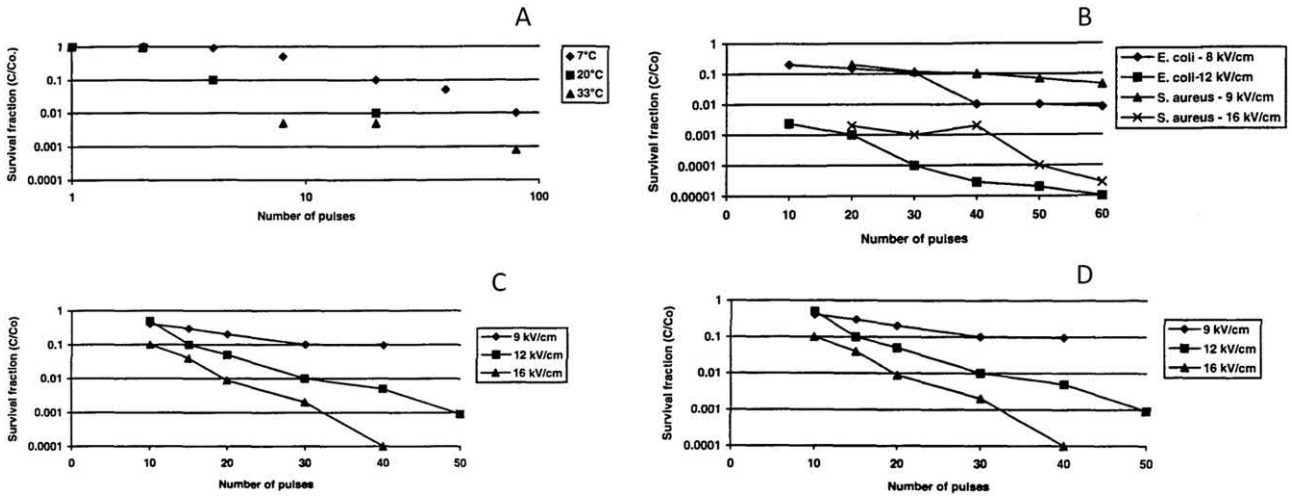

FIGURE 48.13 (A) Effect of temperature on PEF inactivation of *E. coli* suspended in SMUF, using exponential decay pulses. (From Pothakamury et al. [46].) (B) Effect of temperature on PEF inactivation of *E. coli* suspended in SMUF, using square wave pulses. (From Pothakamury et al. [46].) (C) Inactivation of *E. coli* and *S. aureus* in SMUF by REF. (From Pothakamury et al. [48].) (D) Inactivation of *L. delbrueckii* suspended in SMUF. (From Pothakamury et al. [48].)

soup to 20°C. Thermal inactivation of *E. coli* requires up to 10 minutes at 61°C when suspended in bouillon [50].

PEF inactivation of B. *subtilis* and *E. coli* decreased almost 2D when the microorganisms were mixed together in pea soup. Figures 48.14B, 48.14C, and 48.14D summarize the inactivation of *E. coli, B. subtilis,* and the mixture of organisms suspended in pea soup and exposed to selected treatment conditions [49]. There is a significant difference in the inactivation levels ($p < 0.05$) between *E. coli* alone and *E. coli* mixed with *B. subtilis.* PEF inactivation of *E. coli* alone reached 6.5D after 30 pulses at 30 kV/cm and flow rate of 0.5 liters/mm, while an inactivation of 4.0D was observed when *E. coli* was mixed with *B. subtilis. B. subtilis* alone had 5.0D when exposed to 33 kV/cm at 4.3 Hz and 0.5 liters/mm, while only 2.0D were observed when mixed with *E. coli* and exposed to 20 pulses at 30 kV/cm, 4.3 Hz and 0.75 liters/mm or 3.5D after 30 pulses. The results for the inactivation of *E. coli* and *B. subtilis* using PEF demonstrate the feasibility of the technology for preservation of foods containing suspended particles and gelatinized starch.

48.3.1.3 Liquid Eggs

High-intensity PEF (26 kV/cm) treatment in continuous flow systems (continuous recirculation and simple pass) inactivates *E. coli* inoculated in liquid egg 6D with a peak processing temperature of 37.2 ± 1.5°C (Table 48.4, Figures 45.15 and 48.16). PEF treatments with 4 μs pulses were more effective than 2 μs pulses (Figure 48.11D and 48.17A), which may be explained by the amount of energy applied to the liquid egg [42]. Figure 48.18 illustrates the effect of energy input in the inactivation of *E. coli,* with energy input (in joules) calculated as follows:

$$\text{Energy/pulse} = 0.5\ C\ V^2$$

where C is the capacitance, 0.5 μF for 2 μs pulses and 1.0 μF for 4 μs pulses; and V is the measured potential across the treatment chamber (15.6 kV). The total energy input (in joules) after n pulses is calculated by

$$\text{Total energy} = n * \text{Energy/pulse}$$

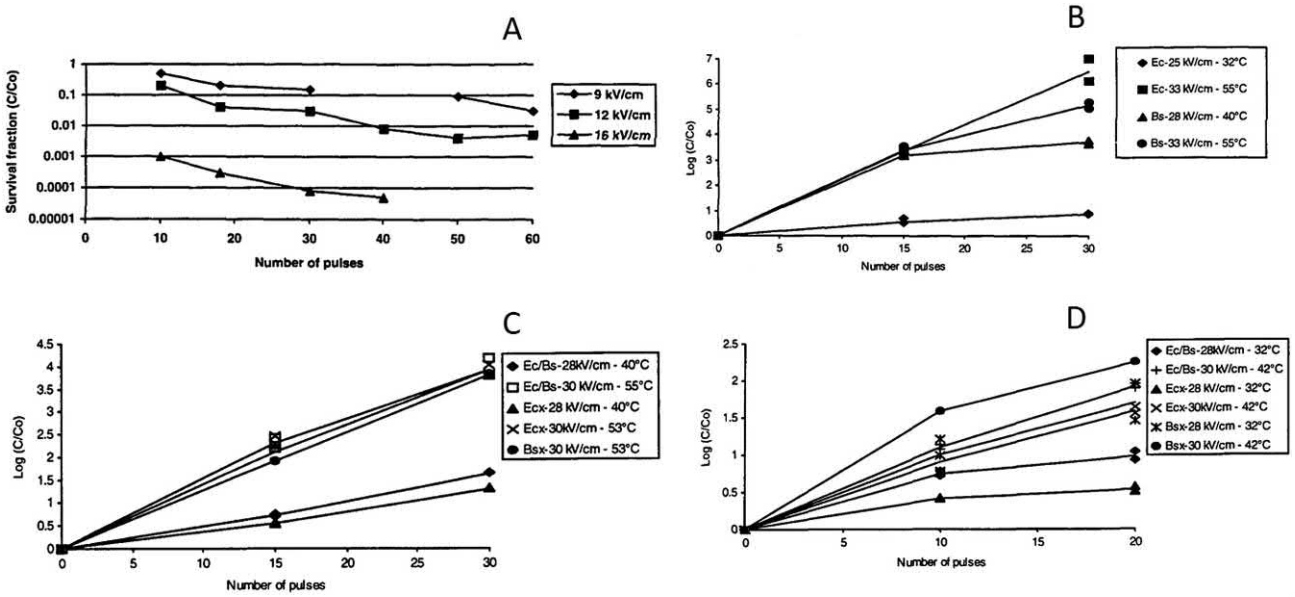

FIGURE 48.14 (A) Inactivation of *B. subtilis* suspended in SMUF. (From Pothakamury et al. [47].) (B) Inactivation of microorganisms suspended in pea soup using PEF at 0.5 liter/min and 4.3 Hz (Ec is *E. coli*; Bs is B. *subtilis*). (From Verga-Mercado et al. [49].) (C) Inactivation of mixture of microorganisms suspended in pea soup using PEF at 0.5 liter/min and 4.3 Hz (Ec is *E. coli*; Bs is B. *subtilis*; Ec/Bs is the overall inactivation for the mixture of microorganisms; Ecx is the inactivation of *E. coli* in the mixtures; Bsx is the inactivation of *B. subtilis* in the mixture). (From Verga-Mercado et al. [49].) (D) Inactivation of mixture of microorganisms suspended in pea soup using PEF at 0.75 liter/min and 4.3 Hz (Ec is *E. coli*; Bs is B. *subtilis*; Ec/Bs is the overall inactivation for the mixture of microorganisms; Ecx is the inactivation of *E. coli* in the mixture; Bsx is the inactivation of *B. subtilis* in the mixture). (From Verga-Mercado et al. [49].)

The survival fraction of *E. coli* in liquid egg is reduced almost 6D with 12,000 J applied in pulses of 4 μs (Figure 48.17A). Grahi et al. [37] nearly reached 5D by exposing *E. coli* suspended in sodium alginate to an electric field of 14 kV/cm with five pulses of 20 μs. Zhang et al. [53] observed a 6D reduction in *E. coli* suspended in potato dextrose agar and exposed to 64 pulses of 40 kV/cm at 15°C and a 9D reduction using 70 kV/cm and *E. coli* suspended in simulated milk ultrafiltrate (SMUF) [54].

Protein, an important nutrient for microbial growth, diminished the effectiveness of the PEF treatment [18, 55]. The inactivation of microorganisms using PEF is more difficult in food materials than in buffer solutions [53]. In general, the bactericidal effect of PEF is inversely proportional to the ionic strength and increases with electric resistivity [5, 26]. The electric resistance of liquid egg (1.9 Q) is low compared to other foods and makes necessary the exposure of liquid egg to a large number (100) of pulses.

TABLE 48.3
Inactivation of an *E. coli–B. subtilis* Mixture Suspended in Pea Soup Using PEF

		28 kV/cm		30 kV/cm	
Flow Rate Frequency	**Number of Pulses**	**Process Temperature**	**Log Reduction (D)**	**Process Temperature**	**Log Reduction (D)**
0.5 liter/min 4.3 Hz	15	43	0.7	55	2.3
	30	39	1.6	55	4.0
0.7 liter/min 6.7 Hz	15	41	0.7	53	4.4
	30	41	0.7	55	4.8
0.75 liter/min 4.3 Hz	10	32	0.8	41	1.1
	20	31	1.0	42	1.0

Source: Verga-Mercado et al. [49].

TABLE 48.4
Treatment Conditions for Liquid Egg Exposed to PEF

Description	Operating Conditions	
	Treatment 1	Treatment 2
Pulse duration (μs)	2	4
Capacitance (μF)	0.5	1
Input voltage (kV)	40	30
Input flow rate (liter/min)	0.5	0.5
Input pulse rate (Hz)	1.25, 2.5	1.25, 2.5
Peak voltage (kV)	15.5	15.5
Peak current (kA)	8.0	8.0
Electric field intensity (kV/cm)	26	26
Pulse energy (J)	60	120
Maximum temperature (°C)	37	37

Source: Martin et al. [42].

There was no significant difference ($p > 0.05$) in the effectiveness of PEF treatment when the pulse rate varied from 1.25 to 2.50 Hz, as the inactivation of E. *coli* in liquid egg was at least 4D if the number of pulses and pulse width remained constant. There was also no significant difference ($p > 0.05$) between the inactivation of *E. coli* using continuous recirculation or stepwise treatments.

48.3.1.4 Apple Juice

Commercial apple juice ultrafiltrated and exposed to different PEF treatments showed no changes in pH, acidity, vitamin C, glucose, fructose, and sucrose content [56] as summarized in Table 48.5. The inactivation of *S. cerevisiae* suspended in apple juice is affected by the intensity of the electric field, treatment time, and number of pulses [51, 57]. Figure 48.19 illustrates the microbial count of S. *cerevisiae* as a function of peak field intensity when two pulses were used and the selected field intensities were 13, 22, 35, and 50 kV/cm. The rate of inactivation increases with an increase in field intensity [51]. Microbial inactivation is a function of the number of pulses, as illustrated in Figure 48.17B. An inactivation of 6D is reported after 10 pulses of 35 kV/cm at 22–34°C. The shelf life of PEF-treated apple juice increases over 3 weeks when stored at either 4°C or 25°C, as illustrated in Figure 48.17C.

48.3.1.5 Skim Milk

48.3.1.5.1 Treatment in a Static Chamber System

PEF treatment inactivates *E. coli* in skim milk at 15°C. The principal parameters influencing the microbial inactivation are the applied electric field intensity and treatment time, which can be expressed by the number of pulses (n) when the width of each pulse is fixed [15]. The *E. coli* survival fraction decreases when milk is treated with an increasing number of pulses at a constant field intensity (Figure 48.17D). The rate of inactivation of *E. coli* increases with an increase in the electric field intensity at a constant number of pulses (Figure 48.20A). Less than one log reduction in *E. coli* population was observed for PEF treatments of 20, 25, and 30 kV/cm and 64 pulses at 15°C. However, PEF treatments at 45 kV/cm, 64 pulses, and 15°C lead to a nearly 3 log cycle reduction [52]. The reported results are consistent with those of Dunn and Pearlman [11], but these authors mentioned that the treatment temperature increased up to 43°C.

Similar *E. coli* inactivation was obtained with 20 kV/cm PEF in saline solution [27]. Hulsheger et al. [21] reduced the population 4 log cycles by applying 20 kV/cm PEF for *E. coli* inoculated in phosphate buffer, and Grahl et al. [37] reached a nearly 5 log cycle reduction by treating *E. coli* suspended in sodium alginate solution with 26 kV/cm PEF. The inactivation

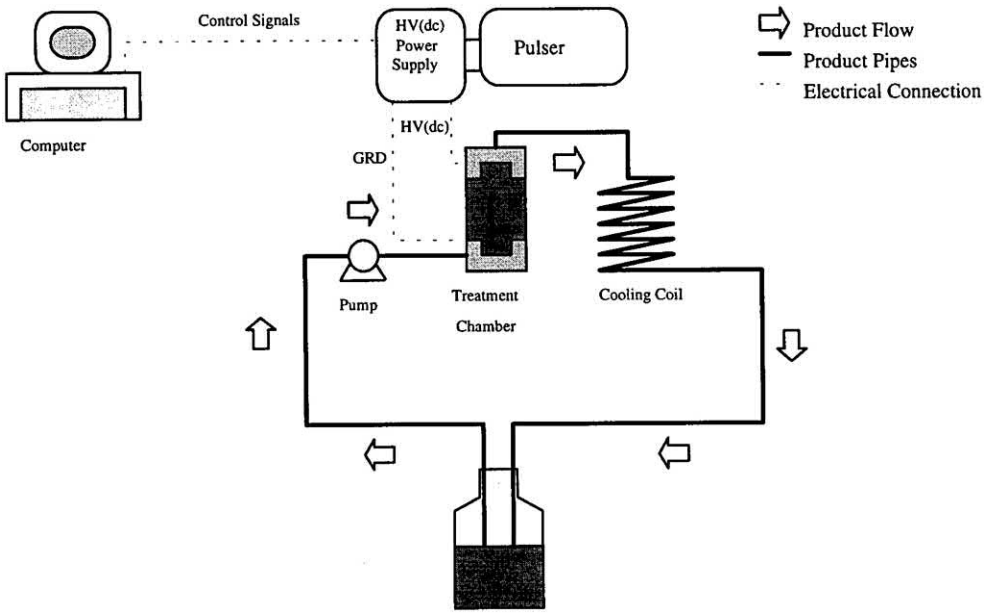

FIGURE 48.15 Continuous recirculation PEF operation.

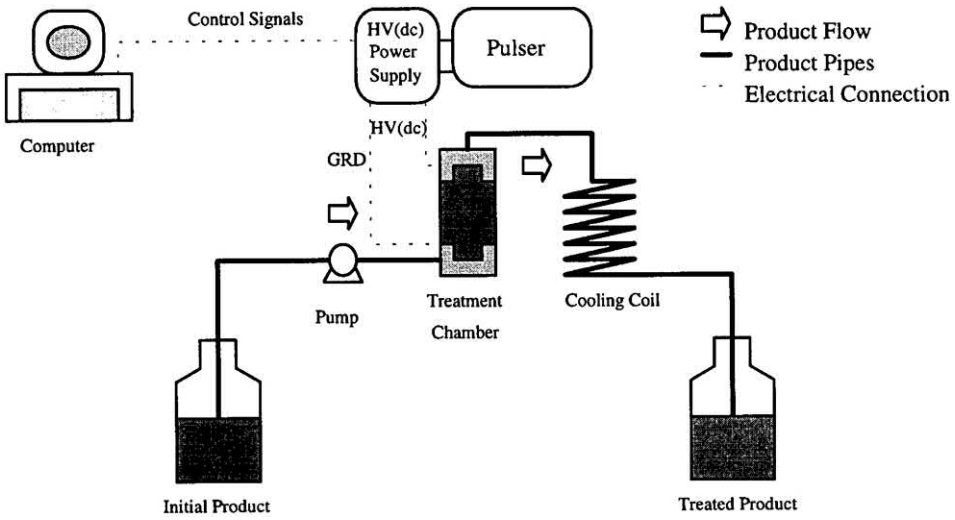

FIGURE 48.16 Single-pass PEF operation.

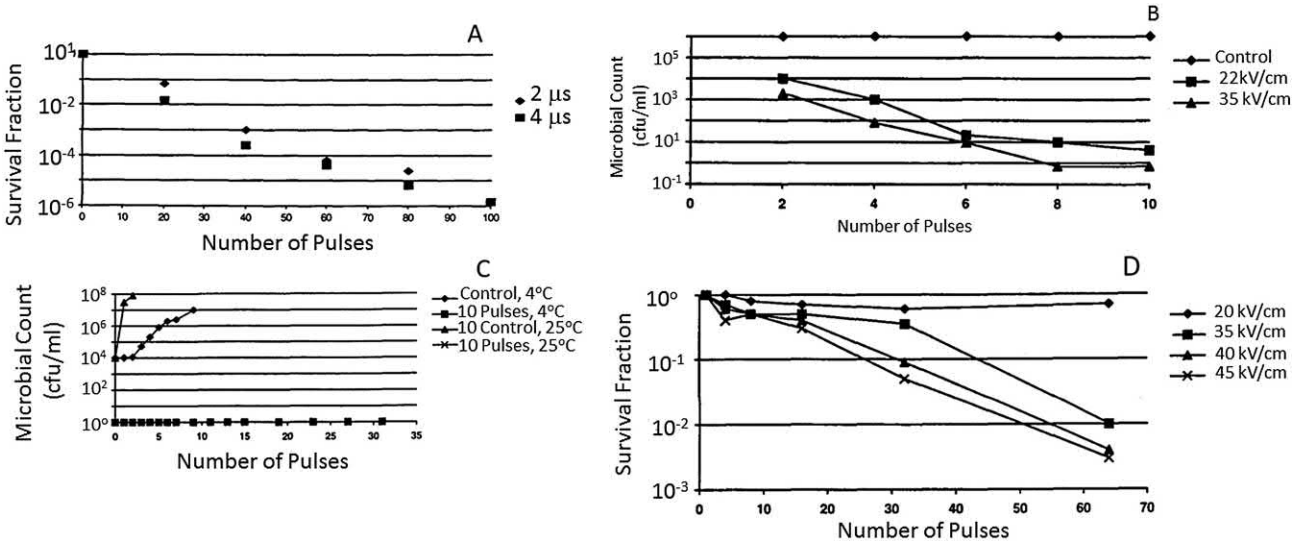

FIGURE 48.17 (A) *E. coli* in liquid egg after PEF treatment at 26 kV/cm and 37°C in a stepwise system. (From Martin et al. [42].) (B) Microbiological count of *S. cerevisiae* in apple juice as a function of the number of 2.5 µs pulses. (From Qin et al. [51].) (C) Shelf life of apple juice after PEF treatment of ten 2.5 µs pulses at 36 kV/cm. (From Qin et al. [51].) (D) Inactivation of *E. coli* in skim milk at 15°C in a static chamber at several field intensities. (From Martin et al. [52].)

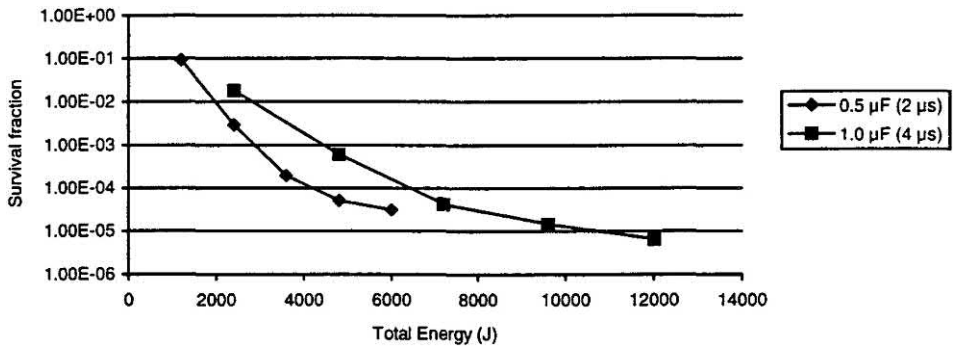

FIGURE 48.18 *E. coli* in liquid egg after PEF treatment at 26 kV/cm and 37°C as a function of input energy. (From Martin et al. [42].)

TABLE 48.5
Apple Juice Chemical Properties before and after PEF

Sample	pH	Acidity (Malic Acid)	Vitamin C (mg/100 g)	Glucose	Fructose	Sucrose
Control	4.10 ± 0.02	2.63 ± 0.02	1.15 ± 0.01	2.91 ± 0.33	4.95 ± 0.64	2.18 ± 0.25
PEF-T1	4.36 ± 0.03	2.67 ± 0.02	1.02 ± 0.02	2.87 ± 0.06	4.96 ±0.11	2.25 ± 0.06
REF-T2	4.18 ± 0.01	2.75 ± 0.07	1.12 ± 0.00	3.01 ± 0.34	5.08 ±0.67	2.21 ± 0.31
REF-T3	4.09 ± 0.01	2.63 ± 0.02	1.02 ± 0.00	2.90 ± 0.09	4.89 ±0.13	2.13 ± 0.06
REF-T4	4.23 ± 0.01	2.61 ± 0.00	1.15 ± 0.24	2.57 ± 0.25	4.33 ±0.47	2.43 ± 0.13

Source: Simpson et al. [56].
Note: The data presented are average values of two experiments each carried out in duplicate.

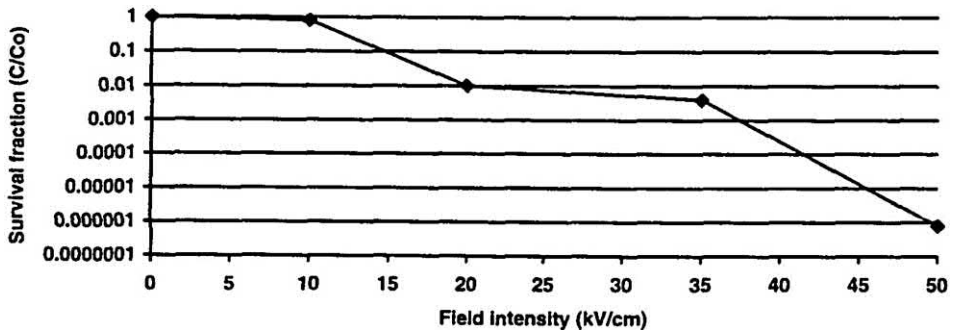

FIGURE 48.19 Survival fraction of *S. cerevisiae* as a function of peak field intensity when two 2.5 μs pulses were applied. (From Qin et al. [51].)

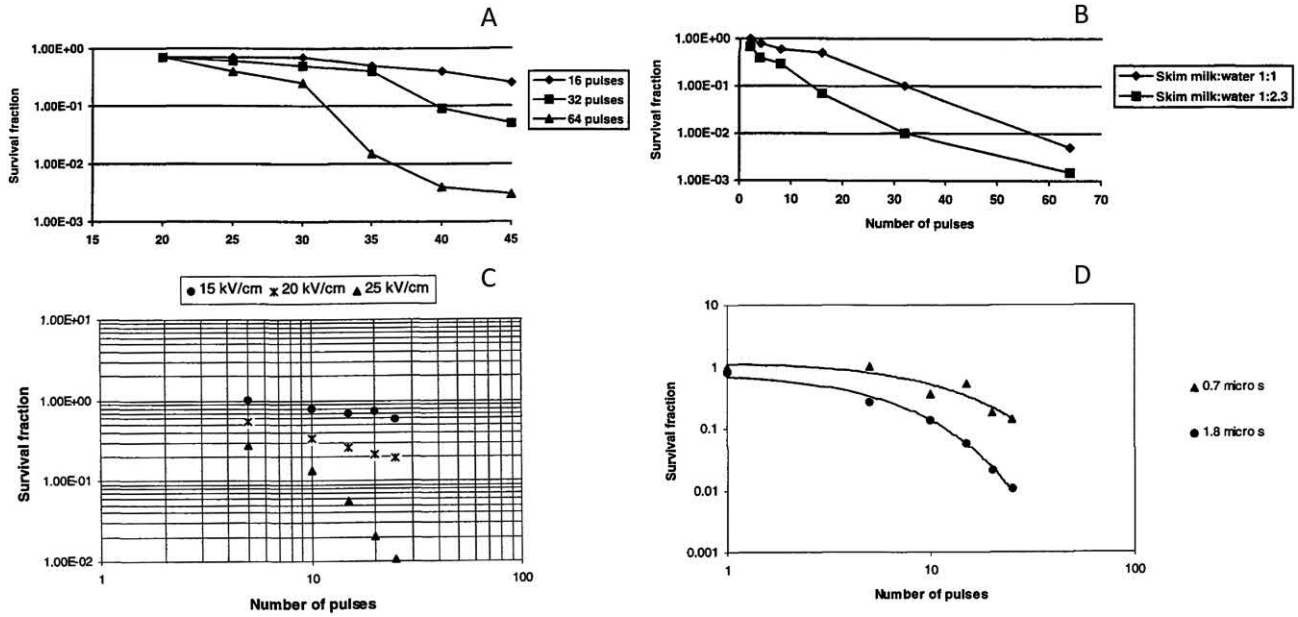

FIGURE 48.20 (A) Inactivation of in skim milk at 15°C in a static chamber with different number of pulses. (From Martin et al. [52].) (B) Effect of skim milk dilution in the inactivation of *E. coli* by 35 kV/cm PEF treatment in a static chamber at 15°C. (From Martin et al. [52].) (C) Inactivation of *E. coli* in skim milk at 15°C in a continuous chamber at different intensities. (From Martin et al. [52].) (D) Effect of pulse duration in the inactivation of *E. coli* in skim milk at 15°C by 25 kV/cm PEF treatment in a continuous chamber. (From Martin et al. [52].)

of *E. coli* in potato dextrose agar by applying 64 pulses of 40 kV/cm at 15°C resulted in a 6 log cycle reduction. Notice that PEF inactivation kinetics in semisolid products are different from the PEF inactivation kinetics in fluids because *E. coli* cells are fixed in a gel matrix, which increases uniformity of inactivation [53]. Inactivation of *E. coli* in skim milk by PEF treatment in a static chamber satisfied Hulsheger's model (Table 48.6) because the destruction of this microorganism in skim milk followed a first-order kinetic for both the electric field intensity and number of pulses.

TABLE 48.6
Kinetics Constant of Hulsheger's Model for *E. coli* Inactivation in Skim Milk by PEF[a]

Electric field Intensity (kV/cm)	Number of Pulses (*n*)	n_{min}	E_c (kV/cm)	*K* (kV/cm)	R^2
35	<64	15.2	—	5.6	0.829
40	<64	13.0	—	6.1	0.958
45	<64	11.0	—	8.0	0.985
<45	16	—	18.7	2.9	0.833
<45	32	—	20.4	3.9	0.861
<45	64	—	19.9	2.7	0.924

Source: Martin et al. [52].
Note: R^2 = correlation coefficient for regression analysis ($p = 0.05$).
[a] Treatment in a static chamber.

TABLE 48.7
Kinetics Constant of Hulsheger's Model for *E. coli* Inactivation in Skim Milk by PEF[a]

Electric Field Intensity (kV/cm)	Number of Pulses (*n*)	n_{min}	E_c (kV/cm)	*K* (kV/cm)	R^2
15	<30	5.4	—	3.9	0.918
20	<30	1.9	—	9.5	0.997
25	<30	2.7	—	5.8	0.955
<30	15	—	13.82	4.3	0.985
<30	20	—	14.62	2.2	0.968
<30	25	—	14.44	2.2	0.938
<30	30	—	12.34	3.5	0.992

Source: Martin et al. [52].
Note: R^2 = correlation coefficient for regression analysis ($p = 0.05$).
[a] Treatment in a continuous chamber.

Martin et al. [52] reported that the minimum number of pulses (n_{min}) necessary to inactivate the microorganisms in skim milk at 45 kV/cm using a static chamber is 11 and 15 pulses at 35 kV/cm, respectively. The critical electric field (E_c) is 19.9 kV/cm with 64 pulses at 45 kV/cm, which is higher than the value reported by Grahl et al. [37] for *E. coli* suspended in sodium alginate solution (14 kV/cm). Zhang et al. [53] calculated 17.5 kV/cm E_c for *E. coli* in semisolid model foods.

It is more difficult to reduce the survival fraction of microorganisms present in skim milk than in buffer solutions and model foods because the composition of skim milk is complex (i.e., high protein content 33–40 g/liter) [58]. These substances diminish the lethal effect of PEF in microorganisms because they absorb free radicals and ions, which are active in the breakdown of cells [18, 55]. Moreover, the inactivation of bacteria by PEF is a function of solution resistance, which is inversely proportional to ionic strength. Survival fractions decrease when medium resistance increases and ionic strength decreases [26, 59]. The measured resistivity of skim milk is 310 Ω cm and that of buffer solutions is even higher. Since dilution of milk increases the resistivity and decreases protein concentration, the effectiveness of PEF treatment is improved. The inactivation rate of *E. coli* suspended in skim milk:water (1:2.3) and exposed to 40 kV/cm in a static chamber at 15°C is higher than when less diluted skim milk (1:1) is used (Figure 48.20B).

48.3.1.5.2 Treatment in a Continuous System

PEF treatment in a continuous-flow chamber also inactivates *E. coli* inoculated in skim milk. An increase in field intensity or number of pulses produces greater bacterial inactivation (Figures 48.20C and 48.21) and microorganism death follows first-order kinetics with both field intensity and number of pulses (Table 48.7). The E_c when PEF treatment was carried out in a continuous system at 30 kV/cm maximum electric field intensity was between 12.34 and 14.62 kV/cm, and n_{min} ranged from 1.9 to 5.4 pulses. These values were lower than those obtained in the same treated product using the static system.

In general, PEF treatment in continuous systems is more effective in terms of microorganism inactivation than in static systems due to the treatment uniformity being greater. Moreover, in this study even though both chambers are of the parallel plate type, the treatment volume in a static chamber is higher (14.5 ml) than the continuous-flow chamber (8 ml). Therefore, the energy density (defined as energy divided by volume) is higher in continuous systems.

The effectiveness of PEF treatment also depends on pulse duration, which increases the *E. coli* inactivation because the energy applied in each pulse is higher. Applying 25 pulses of 0.7 μs each at 25 kV/cm in a continuous-flow chamber reduces the survival fraction of *E. coli* inoculated in skim milk less than one log cycle, but a treatment in the same chamber with the same number of pulses and field intensity and a 1.8 μs duration pulse reduces the survival fraction by more than 2 log cycles (Figure 48.20D).

48.3.2 Denaturation of Proteins

48.3.2.1 Alkaline Phosphatase

The activity of alkaline phosphatase (ALP) in pasteurized milk products has public health significance since the presence of active ALP indicates inadequate pasteurization or cross-contamination with raw milk [60]. In fresh raw milk, ALP is present in association with the membrane of fat globules; in skim milk, it is in the form of lipoprotein particles.

The inactivation of ALP by PEF is a function of the field intensity, the fat content of the milk, and the concentration of ALP. The activity of ALP decreases with an increase in field intensity [60]. A reduction of 43–59% in ALP activity is reported when the enzyme is suspended in 2% milk and exposed to 70 pulses of 0.40–0.45 ms at 14.8–18.8 kV/cm (Figure 48.22A). Seventy pulses of 0.74 msec of a field strength of 22 kV/cm applied to 2 mg/mi ALP in SMUF reduced the ALP activity by 65% (Figure 48.21B). The activity of ALP dissolved in UHT-pasteurized 2% and 4% milk

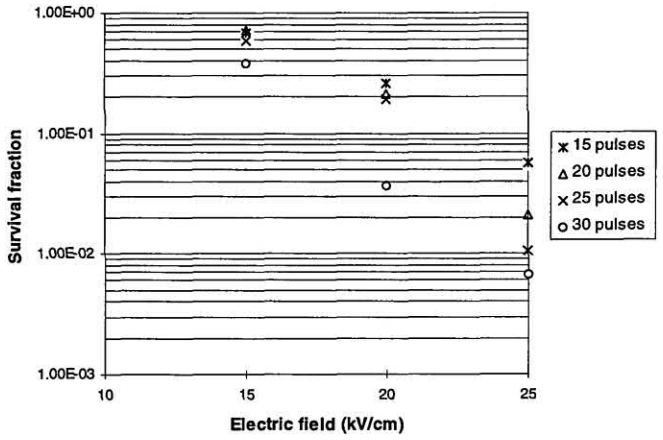

FIGURE 48.21 Inactivation of E. *coli* in skim milk at 15°C in a continuous chamber with different number of 1.8 ms pulses. (From Martin et al. [52].)

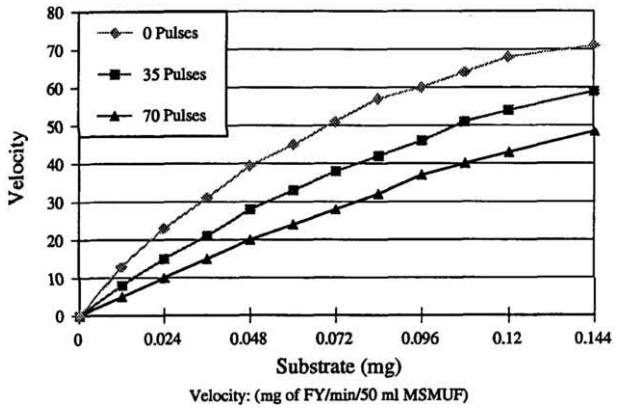

FIGURE 48.23 Initial velocity of fluoroyellow (FY) producing reaction of ALP in MSMUF treated with 0.78 ms pulses of 22.3 kV/cm.

was reduced 59% when exposed to 70 pulses of 0.40 msec at 18.8 kV/cm, while a 65% reduction was observed in nonfat milk as illustrated in Figure 48.22C. ALP suspended in milk (1 ml raw milk in 100 ml 2% milk) using 13.2 kV/cm and 43.9°C after 70 pulses showed a reduction of 96% in activity, whereas heat treatment at 43.9°C for 17.5 min showed only a 30% reduction (Figure 48.22D). Castro [60] demonstrated a reduction in initial velocity of fluoroyellow production of ALP as a function of number of pulses, as illustrated in Figure 48.23. Castro also found that PEF-treated ALP is more susceptible to trypsin proteolysis (70 pulses of 0.78 msec at 22.3 kV/cm), as illustrated in Figure 48.24. The inactivation of ALP is attributed to conformational changes induced by PEF [31, 60].

48.3.2.2 Plasmin and a Protease from *Pseudomonas fluorescens* M3/6

The proteolytic enzyme plasmin and a protease from *Pseudomonas fluorescens* M3/6 were also inactivated using pulsed electric fields. A 90% inactivation of plasmin activity was observed during 30 and 45 kV/cm, 10–50 pulses of 2 μs duration, and a process temperature of 10°C and 15°C [60] as presented in Figures 48.25A and 48.25B. Meanwhile, 80% inactivation was found for a protease extracted from

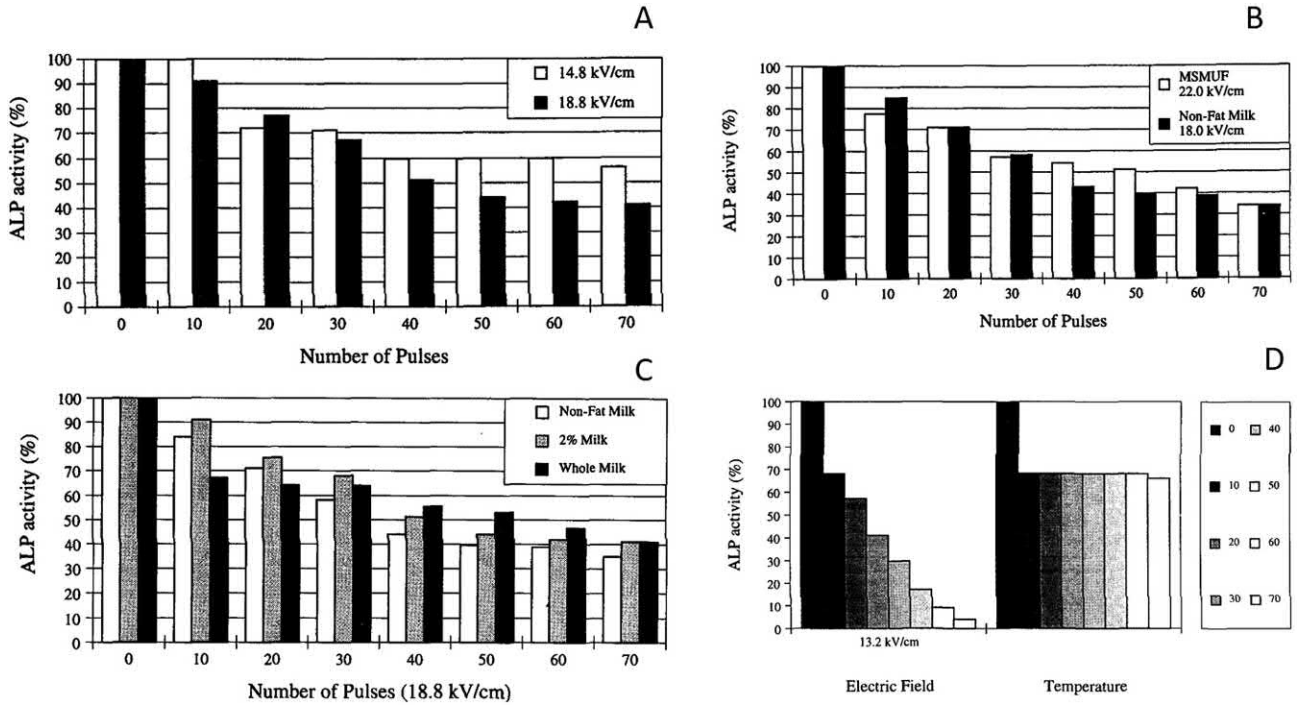

FIGURE 48.22 (A) PEF inactivation of ALP diluted in UHT pasteurized 2% milk. (From Castro [60]) (B) PEF inactivation of ALP diluted in MSMUF or nonfat milk. (From Castro [60].) (C) PEF inactivation of ALP diluted in UHT pasteurized nonfat, 2% and whole milk. (From Castro [60].) (D) Inactivation of alkaline phosphatase by PEF or heating at 44°C for 17.5 minutes. (From Castro [60].)

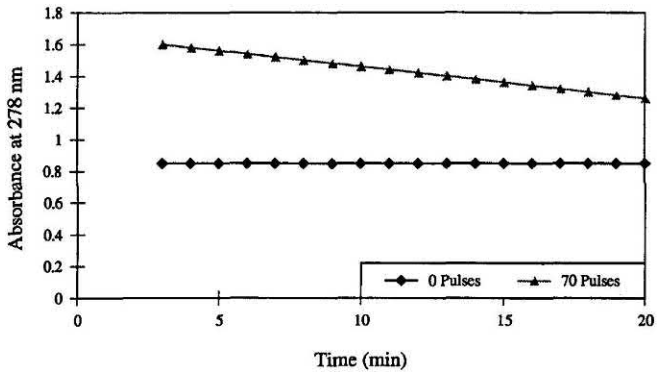

FIGURE 48.24 Trypsin digestion of native and PEF treated alkaline phosphatase. (From Castro [60].)

P. fluorescens when dispersed in Tryptic Soy Broth and exposed to 20 pulses of 2 μs at 11–18 kV/cm and 20–24°C. A 60% inactivation was detected when inoculated in sterilized skim milk and exposed to 98 pulses of 2 μs at 15 kV/cm and 50°C (Figure 48.25C); no inactivation was detected when inoculated in a sterilized casein-Tris buffer and exposed to a PEF treatment similar to that for skim milk. The decreased effectiveness of PEF in the inactivation of the protease in skim milk and the casein-Tris buffer may be attributed to a protective role of the substrate (i.e., casein) against conformational changes of the enzyme induced by the electric fields [61].

The susceptibility of casein to proteolysis varies as a function of treatment conditions [61]; a HIPEF treatment of 25 kV/cm at 0.6 Hz and 30°C was found to increase the proteolytic

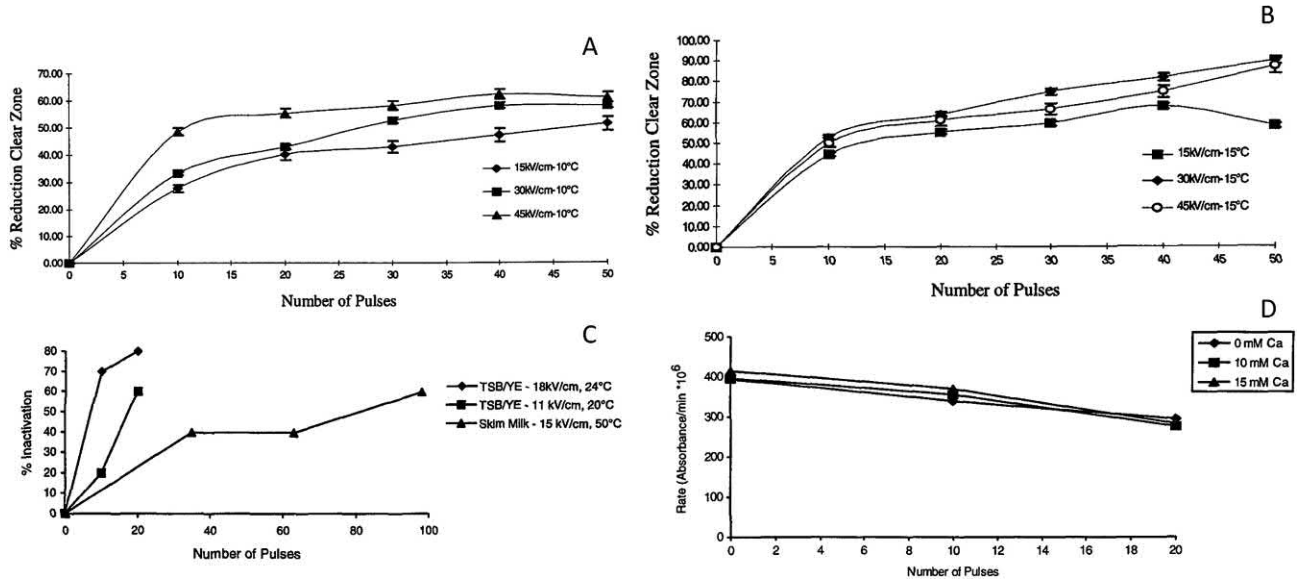

FIGURE 48.25 (A) PEF inactivation of plasmin at 10°C. (From Verga-Mercado et al. [62].) (B) PEF inactivation of plasmin at 15°C. (From Verga-Mercado et al. [62].) (C) Inactivation of a protease from *P. fluorescens* M3/6 in Tryptic Soy Broth enriched with yeast extract (TSB/YE, pulsing rate of 0.25 Hz) and skim milk (pulsing rate 2 Hz) using 2 μs pulses. (From Verga-Mercado et al. [61].) (D) PEF inactivation of protease from *P. fluorescens* M3/6 at 6.2 kV/cm. (From Verga-Mercado [63].)

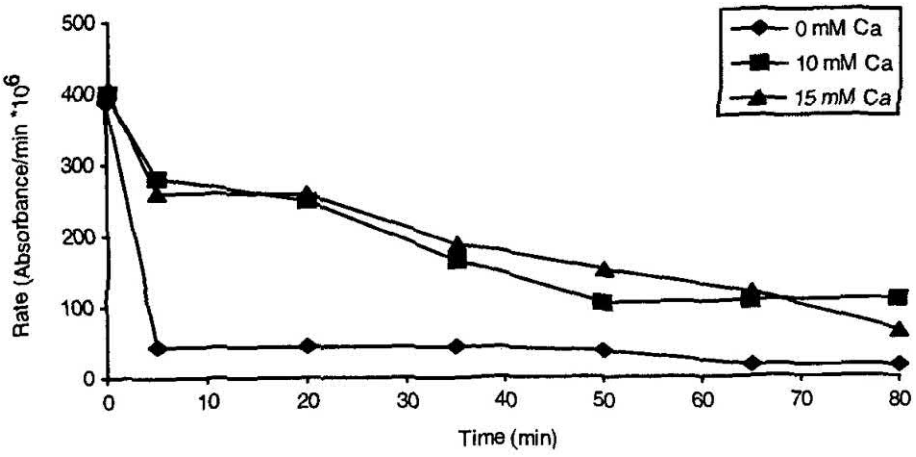

FIGURE 48.26 Thermal inactivation of protease from *P. fluorescens* M3/6. (From Verga-Mercado [63].)

activity in skim milk inoculated with a protease from *P. fluorescens* M3/6. However, 14 or 15 kV/cm at 1 or 2 Hz and 30°C had no significant effect on the susceptibility of casein in skim milk proteolysis, and no significant change was observed in the susceptibility of casein suspended in a casein-Tris buffer when exposed to treatment conditions similar to those for skim milk [61].

The inactivation of the protease from *P. fluorescens* M3/6 when exposed to PEF does not depend on the presence of calcium in the media containing the protease (Figure 48.25D). The inactivation is the same for the three solutions containing 0, 10, or 15 mM calcium. The proteolytic activity of the protease was reduced 30% after exposure to 20 pulses of 700 μs at 6.2 kV/cm and 15–20°C [63].

In contrast to PEF, thermal inactivation of the protease suspended in SMUF does vary with calcium content. Heated samples containing either 10 or 15 mM calcium retained 71% of the original activity compared to 12% retention on samples without calcium after 5 min of heating, followed by a steady decrease in activity as a function of the heating time (Figure 48.26). The analysis by HPLC using the hydrophobic interaction column (HIC) of PEF (20 pulses, 15 mM Ca^{2+}) and heat-treated (5 min, 15 mM Ca^{2+}) samples showed differences in the retention time and peak high of the eluted protein when compared to nontreated samples (Table 48.8). EDTA has a significant inhibitory effect on the proteolytic activity of the protease (Figure 48.27). This result is similar to reported data for the protease from *P. fluorescens*. PEF treatment of samples containing EDTA enhanced the inactivation of the protease in SMUF (Figure 48.28).

TABLE 48.8
Hydrophobic Changes of Protease Suspended in SMUF Induced by PEF and Thermal Treatments

Sample	Retention Time (min)	Peak (mm)
Control	6.01	22.9
20 pulses[a]	5.96	25.4
Heat-treated[a]	5.93	20.6

Source: Verga-Mercado [63].
[a] 15 M Ca^{2+}.

48.4 FINAL REMARKS

The research on pulsed electric fields as a nonthermal process needs to include not only the inactivation of microorganisms, but the inactivation of enzymes, retention of vitamins, and the effect of PEF treatments on other food components. The reported inactivation of enzymes, as well as the increased

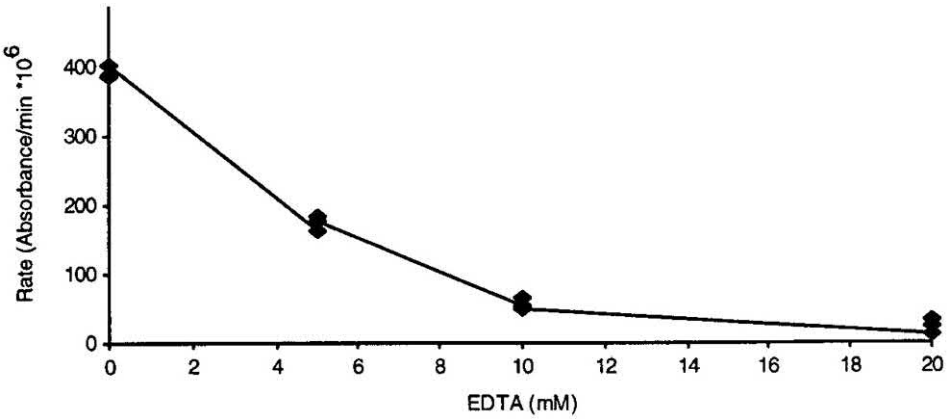

FIGURE 48.27 Inhibitory effect of EDTA on a protease from *P. fluorescens* M3/6. (From Verga-Mercado [63].)

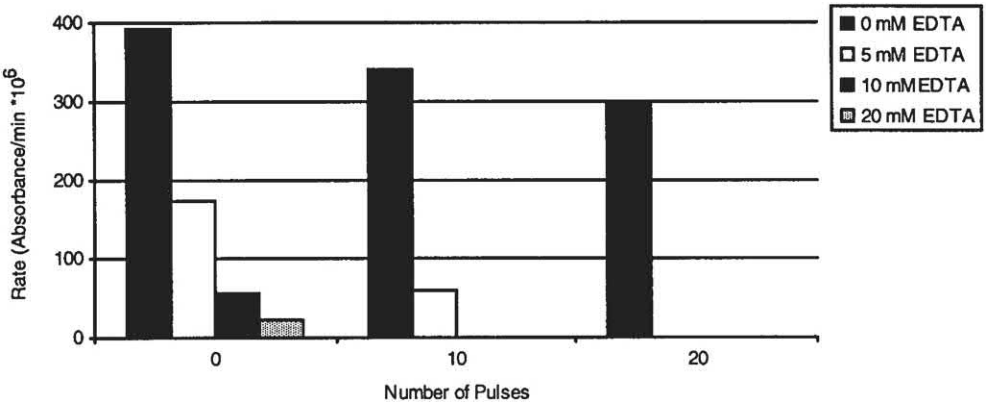

FIGURE 48.28 PEF inactivation of a protease from *P. fluorescens* M3/6 in SMUF with EDTA. (From Verga-Mercado [63].)

proteolysis of casein following exposure to PEF, suggests that detailed research is needed in areas other than preservation. Pulsed electric fields could be utilized as an effective hurdle when used in combination with other preservation factors such as pH and water activity, or as a complementary step with mild thermal processes.

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49 High-Pressure Preservation of Foods

Enrique Palou, Aurelio López-Malo, Gustavo V. Barbosa-Cánovas, Barry G. Swanson, and Mohammad Shafiur Rahman

CONTENTS

49.1 INTRODUCTION

The food characteristics that must be attained in response to modem consumer demands are more freshness or fresh-like; fewer preservatives; less heat and chill damage; less acid; and less salt, sugar, and fat as well as foods without extreme treatments [1, 2]. Safety and quality of foods should be based on substantial improvements in traditional preservation methods or the use of "emerging technologies." One "new" or emerging technology receiving a great deal of attention is high-pressure (HP) processing. Studies examining the effects of high pressure on foods date back to the end of the 19th century, but renewed research and commercialization efforts worldwide could place HP-treated foods in several markets [3–6]. In April 1990, the first high-pressure product, a high-acid jam, was introduced to the Japanese retail market. In 1991, yogurts, fruit jellies, salad dressings, and fruit sauces were also introduced, and two Japanese fruit juice processors installed semicontinuous high-pressure equipment for citrus juice processing [7]. The unique physical and sensory properties of food processed by HP technology offer new chances for food product development, such as minimally processed or raw meat and fish, long shelf-life convenience foods with fresh and natural colors, new types of food gels, and frozen foods with improved quality [8].

At the deepest point in the ocean, the pressure is about 100 MPa and at the center of the earth it is 360 GPa [9]. In commercial applications, the highest pressure used is around 5–6 GPa, which is applied for diamond grit production [9]. High-isostatic-pressure technology is the application of pressure uniformly throughout a product, and it is essentially applied for isostatic pressing, quartz growing, chemical reactors, and simulators [10]. Quartz crystals are grown from a strong alkaline solution of sodium hydroxide at a pressure of up to 200 MPa and a temperature of up to 420°C. Some chemical reactions are carried out at high pressure to increase the yield of the reaction. For example, low-density polyethylene is synthesized at a pressure of 200 MPa and a temperature of 350°C. High-pressure vessels are also used as simulators to test equipment that would be used in a high-pressure environment, e.g., deep in the ocean [11]. The food industry employs the technique of isostatic pressing for applying high pressures to foods.

49.2 ADVANTAGES AND DISADVANTAGES OF HIGH PRESSURE

Figure 49.1 describes the factors affecting the selection of a technology for food processing. There are advantages and disadvantages of high-pressure processing in foods. Therefore, the selection of high pressure needs to be decided considering its advantages and disadvantages when applied to a specific food product. The advantages are (i) it operates at a low temperatures as compared to the conventional thermal treatments, thus retains nutrients, sensory, and improves food quality [12, 13]; (ii) its treatments are independent of product size and geometry, and their effect or action on foods is uniform and instantaneous [14–17]; and (iii) it is useful when other methods are impossible to use, for example, commercial pasteurization or sterilization of nonacidic foods is very difficult or impossible without using some additional factors or methods to enhance the inactivation rate.

49.3 PRINCIPLE AND OPERATION OF HIGH PRESSURE

The basis of high hydrostatic pressure is the Le Chatelier principle, according to which any reaction, conformational change, or phase transition is accompanied by a decrease in volume (i.e., high pressure), while reactions are inhibited by an increase in volume (i.e., low pressure) [5, 18]. With the principle of isostatic processing, as shown in Figure 49.2, the food product is compressed by uniform pressure from every direction and then returns to its original shape when pressure is released [19].

Pressure is an important thermodynamic variable and can affect a wide range of biological structures, reactions, and processes [8]. Pressure primarily affects the volume of a system [20]. The influence of pressure on the reaction rate may be described by the transition state theory: the rate constant of a reaction in a liquid phase is proportional to the quasi-equilibrium constant for the formation of the active reactants [20, 21]. Based on this assumption, it was reported that at constant temperature, the pressure dependence of the reaction velocity constant (k) is due entirely to the activation volume of the reaction (ΔE_v) [20–22]:

$$\left(\frac{\delta \ln k}{\delta P}\right)_T = -\left(\frac{\Delta E_v}{RT}\right) \tag{49.1}$$

where P is the pressure, R is the gas constant (8.314 cm^3 MPa $K^{-1}mole^{-1}$), and T is temperature (K). Water is the most important food ingredient in many food products, thus, its

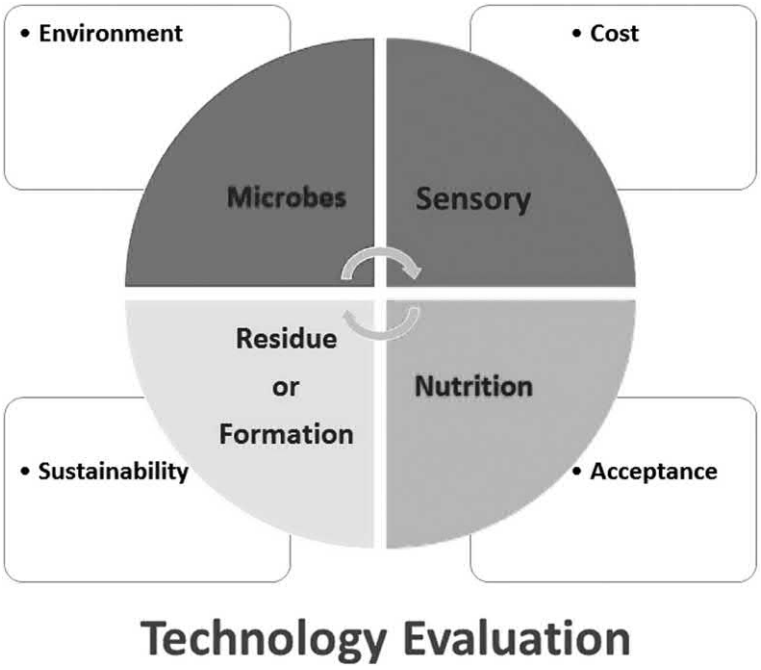

FIGURE 49.1 Factors affecting any technology to be used in food processing.

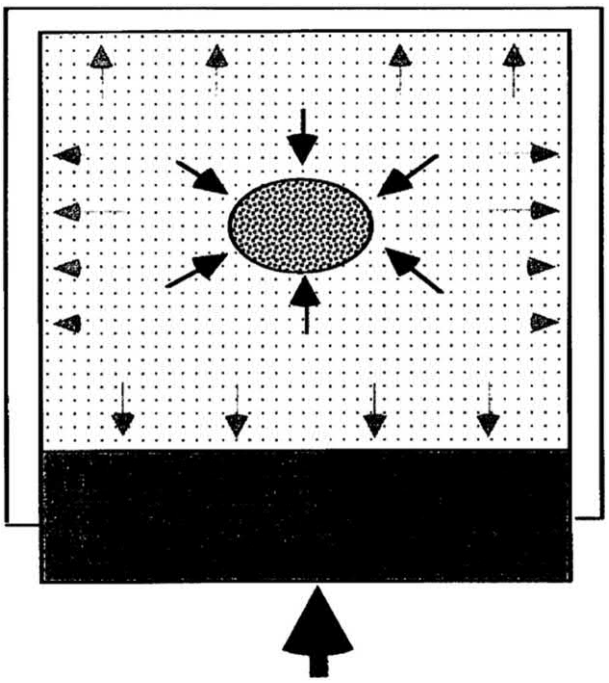

FIGURE 49.2 The principle of isostatic processing. (Adapted from Olsson [19].)

characteristics under pressure are very important. Compared to gases, water is nearly incompressible; adiabatic compression of water increases the temperature by about 3°C per 100 MPa [22]. Self-ionization of water is also promoted by high pressure. The water-freezing characteristics can be changed by the application of pressure [23]. At approximately 1000 MPa, water freezes at room temperature, while the freezing point decreases to –22°C at 207.5 MPa. This event promotes opportunities for subzero storage of foods without ice crystal formation, fast thawing of frozen foods by pressurization, and increasing food freezing by decompression of pressurized foods held below 0°C [8]. In an aqueous system, water molecules surrounding an ionized group align themselves according to the influence of the electrostatic charge, giving a more compact arrangement. Ionization of the acidic or basic groups found in many biomolecules, such as proteins, involves a volume decrease, therefore, chemical reactivity is enhanced by higher pressure [4, 24]. Microorganisms, chemical, biochemical, and enzymatic reactions, as well as some functional properties of biomolecules are affected, to some extent, by high pressure.

49.3.1 Components of High-Pressure Process

A high-pressure system consists of a high-pressure vessel and its closure, pressure-generation system, temperature-control device, and material-handling system [25]. Once loaded and closed, the vessel is filled with a pressure-transmitting medium. Air is removed from the vessel by means of a low-pressure fast-fill-and-drain pump, in combination with an automatic deaeration valve, and high hydrostatic pressure is then generated. High pressures can be generated by direct or indirect compression or by heating the pressure medium [10].

The pressure vessel is the most important component of high-hydrostatic-pressure equipment. Crossland [9] mentioned several considerations that must be taken into account in vessel design: it is necessary to design the high-pressure vessel to be dimensionally stable in a safe-fail way. The vessel does not yield in service; if it fails it should fail by leaking before fracturing. There is also the problem of fatigue, which at the highest pressure cannot be avoided, but an acceptable economic lifetime must be achieved. It is also necessary to establish the minimum number of cycles to failure in order to determine the desired frequency of inspection.

49.3.2 Equipment and Operations of High Pressure

Direct compression is generated by pressurizing a medium with the small diameter end of a piston (Figure 49.3). The large diameter end of the piston is driven by a low-pressure pump. This direct compression method allows very fast compression,

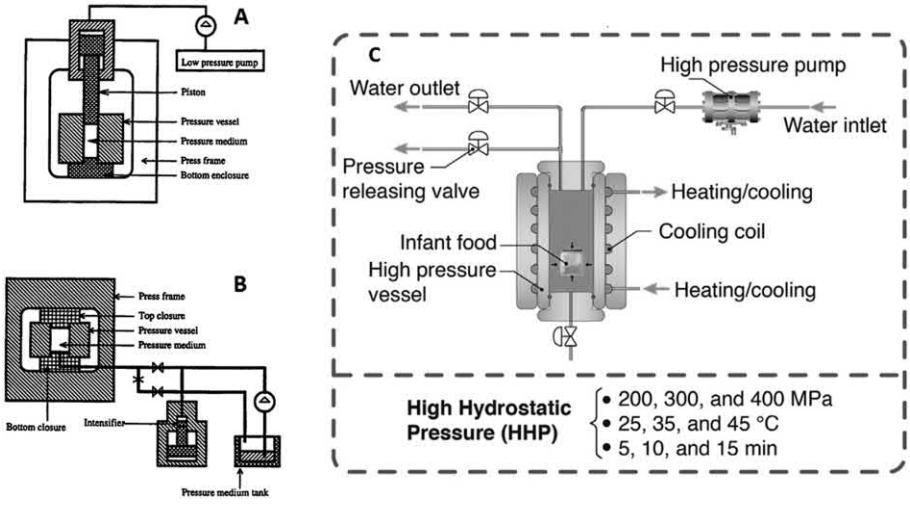

FIGURE 49.3 High pressure system. (A) Direct compression of the pressure transmitting medium, (B and C) Indirect compression of the pressure transmitting medium. (Adapted from Barbosa-Cánovas et al. [11]; Kultur et al. [80].)

but the limitations of the high-pressure dynamic seal between the piston and the vessel internal surface restrict the use of this method to small-diameter laboratory or pilot plant systems.

Indirect compression uses a high-pressure intensifier to pump a pressure medium from a reservoir into a closed high-pressure vessel until the desired pressure is reached (Figure 49.3). Most industrial isostatic pressing (cold, warm, or hot) systems utilize the indirect compression method.

Heating of the pressure medium utilizes expansion of the pressure medium with increasing temperature to generate high pressure. Heating of the pressure medium is therefore used when high pressure is applied in combination with high temperature, and it requires very accurate temperature control within the entire internal volume of the pressure vessel [11]. The isostatic pressing systems may be operated as cold isostatic, warm isostatic, or hot isostatic systems [26] depending on the application.

Cold isostatic pressing is essentially a forming technique used in the metal, ceramics, carbon, graphite, and plastic industries. Powdered materials are filled in an elastomer mold and subjected to high pressure. High-pressure machines work at ambient temperature and use as pressurization fluid a liquid such as water, emulsified water, or oil. Applied pressure is in the range of 50–600 MPa. The cold isostatic pressure process uses "wet bag" or "dry bag" configurations. In the wet bag method, the mold is filled outside the pressure vessel. The mold is then placed in the pressure vessel, which is filled with the pressure medium. In the dry bag method, the mold is fixed in the pressure vessel and separated from the pressure medium by an elastomer tool [11]. The cycle time in a wet bag method is a few minutes, whereas the cycle time in a dry bag method varies between 20 and 60 seconds. Cold and warm isostatic pressure systems are most similar to future food applications. Both the dry bag (in bulk) and wet bag (in container) process options are of interest for food processing [25]. The cold isostatic pressing equipment originally developed for ceramic application was modified to meet additional requirements for food processing. Although the pressure medium used in the vessel is water containing an anti-rusting agent or synthetic oil to protect the pressure vessel against corrosion, food processing requires use of potable water or emulsified potable water [11].

Warm isostatic pressing is also a forming technique. Isostatic pressure is applied in combination with temperatures between ambient and 250°C. A warm isostatic pressure system is used in situations where a chemical reaction develops during pressurization. Hot isostatic pressing is used primarily in the metallic and ceramic industries. The material is uniformly heated and pressurized. The temperature employed is as high as 2000–2200°C, while pressure is 100–400 MPa. The pressure medium used is a gas such as argon, nitrogen, helium, or air. The cycle time typically varies between 6 and 12 hours [10].

49.3.3 Processing Parameters

The processing cost depends on the time of exposure. Processing at 400 MPa with a holding time of 10 minutes is twice as expensive as processing at 800 MPa with no hold time [19]. The combination of pressure, time, and temperature at which the product is processed must therefore be evaluated carefully. A low maximum operating pressure can cause drastic reductions in the fabrication costs. High-pressure processing may be combined with moderately high temperatures, so the operating pressures required are not extremely high [27]. Figure 49.4 shows the pressure and temperature range used for pasteurization and sterilization.

A sterile container filled with food is sealed and placed in the pressure chamber for pressurizing. Ethylene-vinyl alcohol copolymer (EVOH) and polyvinyl alcohol (PVOH) films are recommended for packaging food for high-pressure treatment [28]. Also, the existing multilayer plastic and some aluminum packages may be used for high-pressure processing.

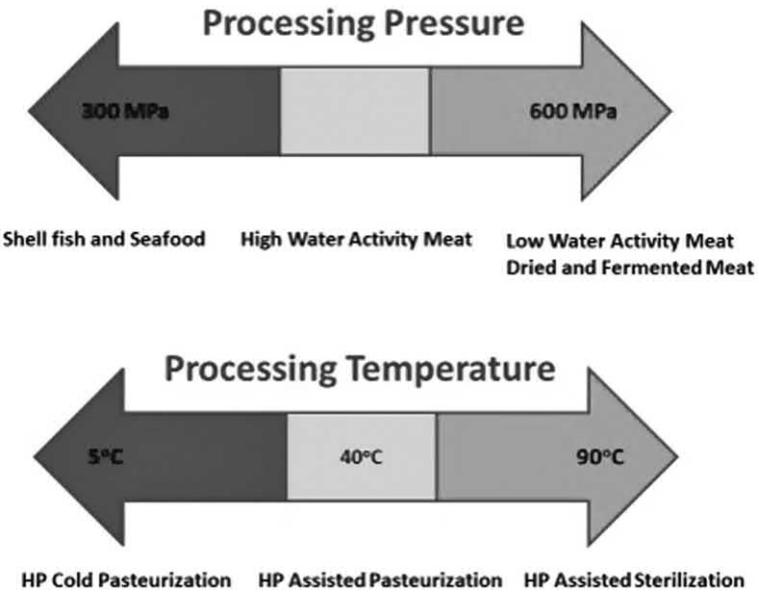

FIGURE 49.4 Pressure and temperature range used for pasteurization and sterilization.

No deformation of the package occurs because the pressure is uniform [29]. The shape of the package needs to be designed to fill the vessel volume as far as possible to increase the economic feasibility of the process. The basis for applying high pressure to foods is to compress the water surrounding the food. At room temperature, the volume of water decreases, as presented in Figure 49.5 [3, 30]. Because liquid compression results in a small volume change, high-pressure vessels using water do not present the same operating hazards as vessels using compressed gases [3].

The capacity of a high-pressure plant depends on three factors: the number of cycles that can be done in a given time, the volume of the product, and the number of high-pressure vessels available. The cycle time is determined by the time needed to handle the food product, including loading, unloading, opening and closing the high-pressure vessel, the pressure holding time, and the pressurization and decompression rates. The productivity of the batch system is increased by a reduction in the pressurizing–decompressing cycle. The pressurizing time is reduced by increasing the delivery rate of the pump [11]. When the required operating pressure is attained, the pumping rate is reduced. At the end of the specified holding time, the pressure vessel is decompressed in two stages to avoid sudden release of pressurized water [31]. Batch processing reduces the risk of large quantities of food becoming contaminated by the lubricants or wear particles from the machinery. Different types of food can be processed in a batch system, without the danger of cross-contamination or the need to clean the equipment after each run [11]. The technical advantage of the batch-type pressure vessel is the simplicity of fabrication when compared to a continuous flow pressure vessel operating at pressures as high as 400–900 MPa [6, 11, 26]. A batch system pressure vessel with a processing capacity of 600 liters/h of liquid food at a maximum operating pressure of 420 MPa was used to commercially produce grapefruit juice in Japan [31]. The production rate of the batch process can be increased by operating pressure vessels in sequence with no lag in the processing times so the system operates sequentially.

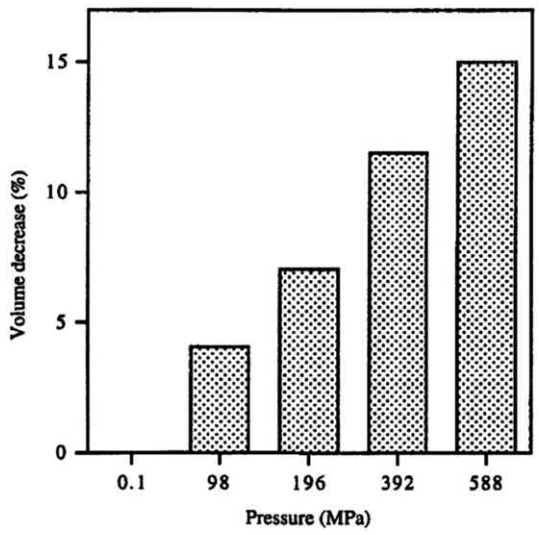

FIGURE 49.5 Decrease in water volume with pressure. (Adapted from Farr [3]).

Food is subjected to high pressure for a specified time period. The holding time in the pressure vessel depends on the type of food and process temperature. At the end of the processing time the chamber is decompressed to remove the treated batch. A new batch of food is placed in the pressure vessel and the cycle begins again [32]. As in heat sterilization of packed products, the water in contact with the product should be of drinking quality, and the lubricating and rust-protecting products should be able to be used in food processing. For bulk processing, the high-pressure equipment should be part of an aseptic line. Parts in contact with the food product should be clean and sterile. The engineering challenges of the application of high pressure in the food industry are primarily the construction of pressure vessels to handle large volumes of food and withstand the high pressures; the pressure vessel should have a short cycle time, be easy to clean, and be safe to operate with accurate process controls. It is desirable to develop a continuous process of pressurization for industrial purposes at reasonably low capital and operating costs [6, 10, 11].

Most of the energy of HP processing is consumed during pressure come-up time when electrical energy is used to achieve the desired pressure by the electric motor in the pump-intensifier systems. At present, the energy required to compress the vessel is mostly lost upon decompression. However, synchronizing the compression and decompression phase in twin-vessel systems could recover half of the decompression energy. In addition, heat could be recovered by chilling or preheating treatment. Maximum product load in the vessel could avoid the loss of compression energy in the transmitting fluid [33].

49.3.4 Commercialization of High Pressure

The Japanese are the leading manufacturers of high-pressure vessels. The major Japanese companies manufacturing high-pressure vessels are Mitsubishi Heavy Industries Ltd., Kobe Steel Ltd., and Nippon Steel Ltd. Other manufacturers of high-pressure equipment include Engineered Pressure Systems, ABB Autoclave Systems Inc., ACB, NKK Corp., Flow International, and Autoclave Engineers [6, 11, 34]. Table 49.1 provides specifications of some commercially available high-pressure systems and some details on pressure vessels and maximum operating pressures. As indicated in the table, the larger the vessel, the lower the maximum operating pressure [6]. The first high-pressure food-processing vessel was manufactured by Mitsubishi Heavy Industries (Tokyo, Japan). The pressure vessels manufactured by Mitsubishi Heavy Industries varied in capacity from 0.6 to 210 liters and the maximum working pressures from 400 to 700 MPa [35]. The increase in the high-pressure unit capacity generally reduces the maximum pressure that can be achieved. To minimize the reduction of equipment life due to repeated use of the pressure vessel, the Mitsubishi pressure vessel is made

TABLE 49.1
Specifications of Selected Commercially Available High-Pressure Vessels

Vendor/Model	Diameter (m)	Length (m)	Volume (L)	Maximum Operating Pressure (MPa)
Mitsubishi Heavy Industries				
MFP 700	0.06	0.2	0.6	700
MCT 150	0.15	0.3	6.0	420
FP-30V	3.00	7.0	50	420
FP-40L	4.00	17.0	210	400
Kobe Steel	0.06	0.2	NA	700[a]
	2.00	3.0	9400	196
ABB Autoclave Systems				
Quintus	0.09	0.225	NA	900[a]
Quintus	0.30	1.250	100	900[a]
Quintus	0.50	2.500	500	900[a]
Engineered Pressure Systems	0.09	0.55	3.5	1380
	0.10	1.0	8.5	1030
	0.10	1.0	37	690
	0.60	2.5	700	550
	0.60	4.5	1250	410
	1.00	4.0	3150	200
	1.70	4.0	9000	100

Source: Pothakamury et al. [6].
NA: Not applicable.
[a] Maximum temperatur≈ 80°C.

up of double cylinders. The inner surface of the pressure vessel is preloaded with high compression stress. The parts of the vessel that come in contact with the pressurizing medium are made of stainless steel. The pressurizing and decompressing cycle is fast. Maximum pressure is attained in 90 seconds. High pressure and a long holding time imply that a great load is applied to the seal of the vessel cover. Self-seal packing with high durability and reliability is used. The seals can withstand repeated opening and closing of the pressure vessel and application of high pressure without leakage. A piston driven by a hydraulic cylinder is used to generate the required pressure [31].

Kobe Steel Ltd. developed a small test pressure vessel and one of the largest pressure vessels available today, with an internal volume capacity of 9400 liters and a maximum working pressure of ~200 MPa. The small test pressure vessel uses a piston for pressurization, and the oil hydraulic system and operational panel are compactly packaged as part of the equipment. Operation is fully automated, and the temperature inside the pressure vessel can be recorded. The equipment also allows the use of a pressure-control program [36].

The research high-pressure food-processing system developed by ABB Autoclave Systems Inc. (Vasteras, Sweden) consists of two components: the process module and the control module. The process module consists of a cabinet, which contains the Quintus prestressed wire-wound pressure vessel, the electrohydraulic pumping system, and a hot water circulation system. The system can reach 900 MPa within 4 minutes. Temperature is maintained by circulating water in channels between the wire winding and the cylinder wall of the pressure vessel. The programmable control module in the ABB high-pressure research vessel monitors and controls the process time, pressure, and temperature. A microprocessor is used to control food loading into the press, press cycling, and downloading to a conveyor [37]. The cost of treating foods in 100- and 500-liter systems is approximately $0.25 and $0.07 per batch, respectively [29]. The high-pressure system Quintus models from ABB Autoclave Systems vary in their dimensions from 0.09 to 0.5 m (internal diameter) and from 0.225 to 2.5 m (internal length) with an internal capacity up to 500 liters and a maximum operating pressure of 900 MPa. Pressure vessels with other dimensions available from ABB Autoclave Systems include 0.045 m diameter × 0.3 m length with a maximum pressure of 1200 MPa; 0.11 m diameter × 0.26 m length with a maximum pressure of 830 MPa; 0.32 m diameter × 1.25 m length (100 liters) with a maximum pressure of 900 MPa; and 0.50 m diameter × 2.50 m length (500 liters) with a maximum pressure of 900 MPa. The fatigue value can be maintained infinitely by replacing a shrunk wear-liner every 30,000 cycles. Changing the liner is convenient and inexpensive. ABB Autoclave Systems is designing and constructing a high-pressure vessel to work in batch mode with a maximum operating pressure of 1700 MPa. The internal diameter of the pressure vessel will be 0.076 m, and the height will be 0.18 m [38].

A warm isostatic pressing system is available from Engineered Pressure Systems Inc. (EPSI), a subsidiary of National Forge Co. (Andover, Massachusetts). The system consists of a double-ended, lined pressure vessel with plug closures. The design parameters of the high-pressure systems constructed by EPSI are listed in Table 49.1. Recently EPSI developed a laboratory-scale pressure vessel with the following specifications: 0.1 m (internal diameter) × 2.5 m (internal height) with maximum operating pressure of 680 MPa and maximum operating temperature of 90°C. While the design pressure is 750 MPa, the maximum operating pressure is 680 MPa. An electrohydraulic intensifier pump with a motor pressurizes the vessel to the operating pressure in 5 minutes or less [39]. An advanced laboratory-scale pressure vessel with a useful diameter of 0.024 m, length of 0.04 m, and maximum pressure of 800 MPa is also available from EPSI.

Current industrial applications of high hydrostatic pressure are presented in Table 49.2. Hayashi [20] presented a list of pressure-processed foods in the Japanese market. The first pressure-processed foods in human history were strawberry, kiwi, and apple jams (Meidi-ya Food Co.). The jams were produced using high-pressure treatment without application of heat. The jams were vivid and natural in color and taste. The list includes fruit sauces and desserts (Meidi-ya Food Co.), mandarin (Wakayama Co.) and grapefruit juices (Pokka Co.), and unrefined rice wine called "nigori-sake." The new sake

TABLE 49.2
Some Commercial High-Pressure-Treated Foods

Product	Processing Conditions	Package	Company
Jams Fruit dressings Fruit sauces Fruit jellies Yogurts	400 MPa, 20°C 10–30 min	Plastic cup (100–125 g)	Meidi-ya
Grapefruit juice	120–400 MPa, 20°C, 2–20min	Glass bottle (200–800 g)	Pokka
Mandarin juice	300–400 MPa, 20°C, 2–3 min	Glass bottle (500 g)	Wakayama
Sugar-impregnated tropical fruits for sherbets and ice creams	50–200 MPa	Paper cups (130 g)	Nisshin
Beef tenderization	100–250 MPa, 20°C, 30 min–3 h		Fuji Chiku & Mutterham
Rice cake	400 MPa, 45–70°C, 10 min		Echigo Seika

Source: Knorr [16,76]; Cheftel [18].

has a white color and fresh flavor, instead of a brown color and cooked smell. Cheftel [18] mentioned that many of the products presented in Table 49.2 are acidic foods, hence they have an intrinsic safety factor. In addition, some of the products are stored and sold refrigerated, consequently, oxidative reactions are retarded. Hayashi [40] reported that at least seven food companies now sell high-pressure-processed foods, including salted raw squid and fish sausages. Knorr [16] mentioned that progress in various additional products is underway, but information is not available due to its confidential nature. In the United States and Europe, developments are being made in fruit products, ready meals, dairy products, meats, fish, and others. Avocado paste (guacamole) is now produced in Mexico using high pressure. Several new industrial applications are expected soon.

49.3.5 Consumer Perception of HP Processing

Consumers' acceptability of a technology is important for its successful applications. Consumers (in the United States, the UK, Europe, and Australia) are more positive toward HP processing due to environmental benefits, improved food safety, and quality [41–45]. European consumers (Slovenia, Hungary, Serbia, Slovakia, Norway, and Denmark) linked HP processing with irradiation and genetically modified organisms and perceived that HP-processed foods have unknown consequences [44, 45]. US consumers are aware and willing to pay additional (39% of respondents) when an explanation for HP and corresponding benefits are given [46]. Chinese consumers could accept the technology, however, consumer education is needed to increase the awareness of the advantages, and specific communication strategies need to be applied [47].

49.3.6 Challenges of HP Processing

Most of the experiments are reported from laboratory- or pilot-scale equipment. Validation from large-scale applications for energy efficiency with reduced wear and abrasion are needed [13]. Commercial feasibility must include research on the design and construction of plant and equipment for the high-pressure processing of foods. Integration of the large amount of available information to design an efficient process is necessary.

Identification of commercially feasible applications is probably the most difficult of the challenges for high-pressure technology [25]. Products processed by high pressure need to have inherent added value or increased profitability due to the expensive process cost. Two important questions need to be answered: Will consumers accept a high-pressure-processed product? Are they prepared to pay an extra cost for a high-pressure-processed food? The commercial application of high-pressure technology in the food industry depends largely on the economic feasibility of the process. The capital cost associated with the equipment purchase and installation is an important obstacle for its commercial implementation [25]. The cost of the high-pressure vessel represents the main fraction of the total cost of an industrial high-pressure processing plant and will depend on the maximum working pressure and the vessel dimensions and processing capacity.

Earnshaw [8] mentioned that it is unlikely that pressure processing will replace canning or freezing; nevertheless, it could find applications for expensive foods with short shelf lives and high-value ingredients such as flavors, vitamins, and functional biopolymers that are heat-sensitive. In Europe, Japan, and the United States there is significant commercial interest in the development of high-pressure food processing, and millions of dollars have been invested in research and development.

High hydrostatic pressure is not a cheap technology, and a systematic approach must be taken to search for processing options to ensure that high-pressure treatments can be successfully and economically applied to a wide range of products [48]. The feasibility studies must include effective equipment design solutions and precisely defined minimum required pressures and time cycles. Continuous operation is also a major task. To optimize high-hydrostatic-pressure preservation techniques, the combined effects of stress factors on the growth of key microorganisms, the storage-dependent changes in food systems after high-pressure treatments, and their shelf-life limiting factors must be clearly understood [49].

49.3.7 Packaging Challenges of HP Processing

The packages should have a sufficient degree of flexibility and resilience to compensate for the reduction in volume [50]. HP treatment produces much less damage than do conventional

thermal treatments in the barrier properties of hydrophilic materials. Gavala et al. [51] found no significant changes in tensile strength of packaging between control and HP-treated samples. The heat seal strength is critical in packages since if a void is present or generated then safety is seriously compromised. In general, thermoplastic materials have stability and can withstand high pressure without significant losses in seal integrity. High headspace volume needs to be avoided since high pressure induced seal damages, delamination and large deformation are observed in flexible packages made of composite materials. The low headspace volume could maximize the use of vessel volume. It is known that gases reduce their volume much more than food or packaging materials, and promotes tensions and failures. In addition, more processing fluid need to be pumped in the vessel for large headspace.

If the polymeric material is layered or coated aluminum, delamination and blistering could be observed since aluminum possesses low compressibility as compared to a polymeric layer. The extent of this effect depends on the pressure exposure time and temperature. In general, the permeability to oxygen, carbon dioxide, and water vapor is not affected by HP treatment. However, damage in the barrier properties is observed in the case of metalized plastic film, especially in the aluminum layer. The reduction of plasticization and subsequent loss of barrier properties occurs at low temperature with HP due to lower water sorption capacity of polymers. HP reduces the voids and free volume of polymer and it causes lower solubility with lower molecular diffusion. These two factors are related to the food–packaging interaction [50, 51]. Packaging must withstand a change of volume up to 18% followed by a return to its original size without losing package and seal integrity and barrier properties (gas permeability, seal and mechanical properties, global migration of packaging components).

49.4 MICROBIAL EFFICACY

The pressure sensitivity of microorganisms varies with the type of microorganism. Gould [1] reports that as high-pressure targets, microorganisms may be divided into those that cause food poisoning and those that cause food spoilage. Microorganisms can be further divided into those that are relatively pressure-sensitive and those that are pressure-resistant. Regarding the pressure sensitivity, the most important categories are the vegetative and spore forms of microorganisms; in general the vegetative forms are inactivated by pressures between 400 and 600 MPa, while spores of some species may resist pressures higher than 1000 MPa at ambient temperatures. Gram-positive bacteria are more pressure-resistant than gram-negative ones [52–54]. Among the gram-positive bacteria, Earnshaw [52] reported that *Staphylococcus* is one of the most resistant and can survive treatment at 500 MPa for more than 60 minutes. Figure 49.6 shows the effect of pressure on microbial lethality.

49.4.1 Vegetative Cells

The relative pressure sensitivity of the vegetative forms of microorganisms has made them the obvious first targets for

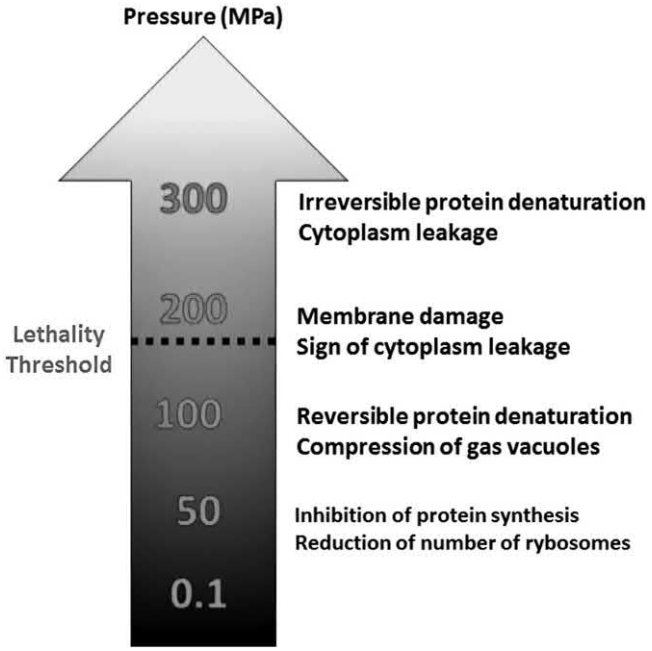

FIGURE 49.6 Level of pressure on microbial lethality. (Adapted from Lado and Yousef [159].)

the preservation of foods by high pressure, and particularly for low pH foods and other foods in which the intrinsic preservation systems already operating ensure that the pressure-resistant food poisoning or spoilage spore-formers that may survive are unable to grow [1]. Increased opportunities must originate when combinations of pressure with some of the other already well-established inhibitory food-preservation techniques are applied using the hurdle technology approach. High-hydrostatic-pressure treatments can be considered as a new hurdle that can be used in combination with other traditional microbial stress factors such as pH, water activity (a_w), and preservatives [55, 56]. However, if high pressure is to be used instead of other stress factors, the kinetics of microbial pressure inactivation must be known as well as the spore resistance of toxigenic bacteria [1]. The extent of microbial inactivation achieved at a particular pressure treatment depends on a number of interacting factors, including type and number of microorganisms, magnitude and duration of high-pressure treatment, temperature, and composition of the suspension media or food [49, 53, 57]. Other experimental variables that must be taken into account include the compression and decompression rates [52].

49.4.1.1 Type and Number of Microorganisms

Zobell [58] reported that most bacteria are capable of growth at pressures around 20–30 MPa; barophiles are organisms that can grow at pressures higher than 40–50 MPa, and those that survive for prolonged periods at pressures >200 MPa are named baroduric or barotolerant. The pressure effects on several pathogenic and spoilage microorganisms inoculated in a pork slurry can be used to illustrate the microbial response to high hydrostatic pressure and the differences among species and microbial forms [54]. *Escherichia coli* counts were almost

unaffected at pressures lower than 203 MPa, but treatments at 304 MPa or higher pressures drastically reduced the initial inocula (10^6–10^7 cfu/g). More than 6 log cycles of *E. coli* were reduced at pressures higher than 405 MPa for 10 minutes. For *Saccharomyces cerevisiae*, less than 2 log cycle reductions were observed at pressures lower than 304 MPa and more than 6 log cycles at pressures higher than 405 MPa. The *Bacillus cereus* spore counts were not reduced considerably (less than 1 log cycle) even in treatments at 608 MPa for 10 minutes.

Patterson et al. [53, 59] reported important information about the effect of high pressure on foodborne vegetative pathogens. The gram-negative bacteria are pressure sensitive [60, 61]. Among these, *Vibrio parahaemolyticus* is one of the most sensible; a 6 log reduction in the initial population can be attained in treatments at 200 MPa for 20 minutes. On the other hand, gram-positive pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* require a 6 log reduction in the initial inoculum treatments 20 minutes at 340 and 400 MPa, respectively [53]. Another pressure-resistant pathogenic bacteria is *E. coli* 0157:H7; Takahashi et al. [61] reported that for a 6 log cycle reduction, the treatment must be at 700 MPa for 13 minutes. The more complex membrane structure of gram-negative bacteria is more susceptible to environmental changes like those caused by high-pressure treatments [54].

Earnshaw [52] reported that little difference was observed in the overall rates of *Staphylococcus carnosus* inactivation for treatments at 650 MPa with different initial populations ranging from 10^7 to 10^3 cfu/ml. When 10^2 cfu/ml were initially inoculated, a faster inactivation rate was observed. It is obvious that with a great number of initially inoculated microorganisms, the effects of specific high-pressure treatments can be observed, but studies with realistic numbers of microorganisms are also needed. The pressure sensitivity of microorganisms may vary with the species and probably with the strain of the same species and with the stage of the growth cycle at which the organisms are subjected to the high-hydrostatic-pressure treatment. In general, cells in the exponential phase are more sensitive to pressure treatments than cells in the log or stationary phases of growth [52, 53, 58, 62]. Isaacs et al. [63] reported that freshly inoculated *E. coli* cultures growing rapidly were more sensitive than *E. coli* cultures that had reached the growth stationary phase. No effect after 7 minutes at 200 MPa with cells in the stationary phase and around *5* log cycle reductions with young cells from the log phase were observed. Also, it has been established that the cell age distribution in inoculation studies might be an important factor in the result obtained after high-pressure treatments. Bacteria in the stationary phase are smaller and more spherical than in the log phase, when they are rapidly growing, rod-shaped, and exhibit an accelerated metabolism. Isaacs et al. [63] stated that the greater resistance to pressure when the cell metabolism slows down may reflect the accumulation of cell components, which can reduce the effects of high pressure.

Considerable variation in pressure sensitivity between strains isolated from different foods or culture collections has been reported for the same microorganisms. Patterson et al. [53] reported a 3 log reduction for *L. monocytogenes* from a culture collection after 30 minutes at 375 MPa. The same pressure treatment reduced the initial population >10^4- and 10^7-fold, for strain Scott A and a chicken isolate, respectively. Cheftel [18] reported that when the pressure resistance of various microorganisms is compared, the survival fractions determined by different investigators vary by a factor of 1 to >8 for different species of the same genus (*Salmonella*) or by a factor of 1.5–3.5 for different strains of the same microorganism (*L. monocytogenes*).

49.4.1.2 Extent and Duration of High-Hydrostatic-Pressure Treatments

Generally, an increase in pressure increases microbial inactivation. However, increasing the duration of the treatment does not necessarily increase the lethal effect. Above 200–300 MPa, the inactivation ratio of vegetative cells increases with pressure or process time [53]. Table 49.3 presents some results about the effects of high pressure level and exposure time on several microorganisms. As mentioned before, the microbial response to high-pressure treatments depends on the type of microorganism. For each microorganism, there is a pressure-level threshold at which no effects of increasing the exposure time are detected. There also exists a pressure level at which increasing treatment time causes significant reductions in the initially inoculated microbial counts. The intrinsic conditions of the suspension media such as pH, a_w, and nutrients may influence the pressure threshold, which can increase or decrease depending on the microorganism and variation of intrinsic, extrinsic, and processing factors. Kinetic studies at pressures over the pressure threshold are needed. With reliable kinetic data, the pressure sterilization or pasteurization of foods can be predicted and achieved.

49.4.1.3 Temperature

The temperature during pressurization can have a significant effect on the inactivation of microbial cells. Several authors [18, 64–67] observed that the resistance to pressure of an endogenous or inoculated microbial strain is maximal at normal temperatures (15–30°C) and decreases significantly at higher or lower temperatures. Freezing temperatures (–20°C) in treatments ranging from 100 to 400 MPa for 20 minutes enhance microbial inactivation when compared with high-pressure treatments at 20°C [61]. Hashizume et al. [68] reported that *S. cerevisiae* cells were more effectively inactivated by high-pressure treatments at elevated (40°C) or subzero (–0 and –20°C) temperatures.

The decrease in pressure resistance of vegetative cells at low temperatures (<5°C) may be due to changes in the membrane structure and fluidity, weakening of hydrophobic interactions, and crystallization of phospholipids [18]. On the other hand, moderate heating (40–60°C) may also enhance the pressure microbial inactivation, resulting in some cases in a lower minimal inactivation pressure [18, 64]. Ogawa et al. [69, 70] reported an enhanced inactivation of natural flora and inoculated microorganisms in mandarin juice treated at 40°C in combination with pressures in

TABLE 49.3
Effect of High-Hydrostatic-Pressure Treatments on Selected Microorganisms

Microorganisms	Substrate or Suspension Media	Treatment Conditions		Decimal Reductions	Reference
Saccharomyces cerevisiae	Satsuma mandarin juice	250 MPa	5 mi≈	2	[69]
			10 mi≈	4	
			30 min	6	
Aspergillus awamori	Satsuma mandarin juice	250 MPa	5 mi≈	3	[69]
			10 mi≈	4	
			30 min	>4	
		300 MPa	5 min	5	
Listeria innocua	Minced beef muscle	330 MPa	10 mi≈	2	[64]
			20 mi≈	3	
			30 mi≈	5	
		360 MPa	5 mi≈	1	
			10 mi≈	2	
			20 mi≈	4	
			30 mi≈	6	
Listeria monocytogenes	10 mM phosphate buffer saline pH 7.0	300 MPa	10 min	<1	[59]
			20 mi≈	1	
			30 mi≈	2.5	
		350 MPa	10 mi≈	4	
			20 mi≈	5.5	
			30 mi≈	6.5	
		400 MPa	10 mi≈	6	
			20 mi≈	8	
			30 mi≈	8	
Vibrio parahaemolyticus	100 mM phosphate buffer pH 7.0 with 3% NaCl	103 MPa	20 min	<1	[60]
			4≈	1	
		138 MPa	10 mi≈	1.5	
			2≈	2	
			3≈	4	
		172 MPa	10 mi≈	2.5	
			2≈	4.5	
			3≈	6	
Salmonella typhimurium	63 mM phosphate buffer pH 7.0	241 MPa	30 min	<1	[79]
		276 MPa	10 min	<1	
			2≈	1	
			3≈	1.5	
		345 MPa	10 mi≈	1.8	
			20 mi≈	2.5	
			30 mi≈	3	
Total plate count	Fresh-cut pineapple	200 MPa	5 min	0.6	[142]
		270 MPa	5 min	1.8	
≈		4°C	15 min	1.6	
		340 MPa	5 min	1.9	
		4°C	15 min	3.0	
			40 min	2.1–2.9	

the range 400–450 MPa. The psychrotrophic bacteria (i.e., *Pseudomonas fluorescens* and *Listeria innocua*) were more sensitive to the effects of pressure at low temperatures, and thermotolerant bacteria (i.e., *Citrobacter freundii*) was more sensitive at 35°C and 50°C. For the three bacteria, the greatest inactivation was obtained when combining the pressure treatment with 50°C [64].

Zobell [58] observed that the maximum microbial growth temperature at increased pressure is generally a few degrees higher than the optimum growth temperature at atmospheric pressure. Sale et al. [71] reported an increasing sensitivity to pressure of *Bacillus coagulans* spores with temperature. Roberts and Hoover [72] evaluated the effect of combinations of pressure at 400 MPa with heat against spores of *B. coagulans* and at 25°C observed less than 1 log cycle reduction in the initial inocula. As the temperature during pressurization increased, the effectiveness of the high-pressure treatment increased; 2 and 4 log cycle reductions were observed at 45°C and 70°C, respectively.

49.4.1.4 Composition of Suspension Media or Food

The effect of pressure on inhibition or inactivation of microorganisms in combination with major environmental variables should be analyzed for a better understanding of the mode of action of this preservation technique. The extent of microbial reduction achieved by a high-hydrostatic-pressure treatment depends on a number of interacting factors including the composition of the media or food [49, 57]. Many food constituents appear to protect microorganisms from the effects of high pressure [4, 6, 49, 53, 57, 73]. Therefore, it is important to evaluate high-hydrostatic-pressure treatments for each individual case.

Dring [74] observed that nonnutritive solutions reduce the microorganism's barotolerance. Marquis [75] reported that an enriched media protects microorganisms against pressure and suggested that free amino acids and vitamins are available and therefore the cells are protected. The pressure resistance of fungi increases as the sugar (sucrose, fructose, and glucose) concentration on the media increases [68, 70, 73].

A baroprotective effect of reduced water activity for organisms that can grow at reduced a~ has been reported [15, 16, 49, 57, 76, 77]. Oxen and Knorr [73] observed that high-hydrostatic-pressure treatment at room temperature and 400 MPa for 15 minutes inactivated the yeast *Rhodotorula rubra* when the a~ of the suspension media was higher than 0.96, while the number of survivors was higher when the a_w was depressed. Similar treatment at 30°C achieved 7 log cycle reductions in the initial inocula at a_w 0.96; 2 log cycles were reduced at a_w 0.94, and no reductions were observed when the a_w of the suspension media was 0.91 [15]. At higher temperatures (45°C) the yeast was inactivated even at low a_w values. Oxen and Knorr [73] demonstrated that pressure–temperature combination treatments result in faster and greater yeast inactivation in comparison with treatments where only temperature or pressure is applied. For heat inactivation, treatments for 15 minutes at 70–80°C and atmospheric pressure were required to inhibit *R. rubra*.

Palou et al. [49] reported the effect of reduced a_w (or increasing soluble solids concentration) on *Zygosaccharomyces bailii* inhibition suspended in laboratory model systems adjusted to pH 3.5. The effect of the model system with soluble solids concentration on the viability of *Z. bailii* without (0.1 MPa) and with a high-pressure treatment of 5 minutes at 345 MPa was studied. *Z. bailii* grew without the pressure treatment in the range of sugar concentration studied (2–59% w/w), rapidly reaching high counts (~$l0^7$ cfu/ml). This yeast is osmotolerant and its growth occurs in media containing up to 60% (w/w) glucose, with a_w 0.85. The soluble solids concentration in the model systems considerably affects the recovery counts after high-pressure treatment, being higher as the soluble solids concentration increased. Greater counts were observed for the experiments with sugar concentrations >40%. In experiments with less than 20% soluble solids, complete inhibition (<10 cfu/ml) of *Z. bailii* was observed. Ogawa et al. [69] reported that for *S. cerevisiae* inoculated in concentrated fruit juices, the number of surviving microorganisms depends on the juice-soluble solids concentration and observed that the inactivation effect at pressure ≤200 MPa decreased as juice concentration increased. Hashizume et al. [68] also reported an increase in the number of surviving *S. cerevisiae* cells with increasing concentrations of sucrose (0–30% w/w) when pressurized at 260 MPa for 20 minutes at 25°C.

Palou et al. [49] reported a linear decreased relationship between the water activity depression factor $(1 - a_w)$ and log of Z. *bailii* survival fraction (N/N_0) after a high-pressure treatment at 345 MPa for 5 minutes. As a_w of the model system decreased, the number of surviving *Z. bailii* increased. The results obtained by Palou et al. [49] without pressure treatment demonstrate that *Z. bailii* was adapted to grow at the selected a_w values, therefore the observed high-pressure effects can be attributed to this variable and not to the effects of reduced a_w on the extension of the lag phase or on the reduction of the growth rate. At $(1 - a_w) > 0.07$, reductions smaller than 1 log cycle were observed [49]. In comparison, Pandya et al. [78] reported for *Z. bailii* suspended in buffer solutions (a_w ~1.0) with pH 4.0, 5.0, and 6.0 that 7 log cycles were reduced in treatments at 304 MPa for 10 minutes, resulting in the total inhibition of the initial inocula. The resistance to inhibition at reduced a_w values may be attributed to cell shrinkage, which probably causes a thickening in the cell membrane that reduces membrane permeability and fluidity [49]. The increased baroresistance of microorganisms at low a_w may also be attributed to a partial cell dehydration due to the osmotic pressure gradients between the internal and external fluids, which may result in small cells and thicker membranes and an increased pressure resistance [15]. The baroprotective effect of reduced a_w reveals that inhibition of microorganisms by high pressure depends not only on pressure level and extent of the treatment but also on the interactions with other intrinsic and extrinsic variables that influence the microbial response [49]. The design of effective pressure treatments that assure microbial stability of foods will depend on an understanding of the relationships between microorganisms and food components.

49.4.1.5 Microbial Cell Recovery after Pressurization

The high-hydrostatic-pressure effects on microorganisms are often determined by plate counts, and usually dilution and plating are made just after treatment. Survival, as measured immediately after pressure release, may differ from that determined after a repair period in the food or in an enriched medium [18]. Recovery after pressure treatment is a very important consideration for process efficacy and death kinetics assessment [53]. Metrick et al. [79] suggested that the lack of nutrients in phosphate buffer prevents the recovery of the pressure-damaged cells. The ultimate fate of the injured cells will depend on the conditions after pressure treatment. However, the fact that pressure can cause injury may be advantageous when high hydrostatic pressure is combined with other preservation methods [53].

The possibility of cell recovery exists, and in many cases, pressure-treated microorganisms may not be detected in plate count methods because of their failure to initiate growth when they are plated immediately after treatment. However, if the repair mechanism remains intact, the microorganisms may be capable of regeneration and growth. Isaacs et al. [63] observed that for *E. coli* suspensions treated at 200 MPa for 0–6 minutes and plated on selective (Mac-Conkey and Eosin Methylene Blue agars) and nonselective (Tryptone Soya agar) media, the survival fraction was greater for bacteria plated on the nonselective agar. This was attributed to the inhibitory ingredients contained in the selective media, indicating that there is a proportion of microorganisms which, after pressurization, can repair and reproduce, whereas the added stress caused by culturing on selective media inhibits the repair process.

Pressure treatments lead to significant reductions in the initial population of *Z. bailii*, but not enough to inactivate the initially inoculated cells, further incubation reveals that the survivors can grow [57]. Yeast cells subjected to physical or chemical hurdles may become injured or sublethally stressed; the recovery of these cells requires generally more incubation time and the injured survivors are capable of growth even in systems with 1000 ppm potassium sorbate and reduced a_w. In treatments rendering complete inhibition of 10^5 *Z. bailii* cfu/ml after 48 hours, the same result was obtained with a longer (120 h) incubation period [57]. Carlez et al. [65] demonstrated that minced meat inoculated with *Pseudomonas* strains treated for 20 minutes at 300 or 450 MPa results in no microbial growth detected after 2 or 6 days of storage, respectively, when stored at 3°C. However, the *Pseudomonas* counts increased with a longer storage. The lag time before reappearance of microbial growth was related to the intensity of the pressure treatment. Carlez et al. [65] defined two different pressure levels: a lower one that causes microbial injury and delays growth, and a higher one that induces complete inactivation of vegetative microorganisms.

Kultur et al. [80] studied the inactivation of total mesophilic aerobic bacteria (TMA) and total yeasts and molds (TYM) during HP processing (pressure: 200, 300, and 400 MPa; temperature: 25°C, 35°C, and 45°C; treatment time: 5, 10, and 15 min) in the case of vegetable-based infant food. Pressure, time, and temperature had a significant influence on both TMA and TYM inactivation depending on the types. Synergistic effects were observed between pressure, time, and temperature in the reduction. HP processing at 400 MPa resulted in a complete inactivation of TMA as well as TYM after 15 min of treatment at 45°C.

49.4.2 Microbial Spores

Bacterial spores have demonstrated pressure resistance [71, 81], and it has been suggested that spore proteins are protected against solvation and ionization. The structure and thickness of the bacterial spore coats are believed to account for this high resistance. Microbial spores suspended in foods and laboratory model systems could be inactivated by high-pressure treatments, but compared with the requirements for vegetative cells the treatment conditions must be extreme: higher pressures and long exposure times at elevated temperatures [82]. Hydrostatic pressure can cause spore germination. Some authors suggested a high-pressure treatment to induce spore germination and a subsequent treatment to inactivate the germinated microorganisms [16, 76]. Gould and Sale [81] studied the germination of *Bacillus* spores and demonstrated that treatments at 25 MPa and 50°C for 30 minutes cause the germination of 50–64% of the initially inoculated spores.

Crawford et al. [83] reported a decrease in viable *Clostridium sporogenes* spores with increasing pressure up to 690 MPa; higher pressures were ineffective in further reducing the spore counts. Crawford et al. [83] postulated that spore germination occurred at pressures lower than 690 MPa and the germinated cells were inactivated by the pressure treatment, leading to a maximum reduction in the initially inoculated spore counts. At higher pressures, a small fraction of the spore population may have remained highly resistant and capable of resisting pressures of 830 MPa. When pressure and temperature were combined at 690 MPa and 80°C for 20 minutes, the treatment was more effective, with a significant reduction in the *C. sporogenes* spore count [83]. Mallidis and Drizou [84] confirmed the extraordinary pressure resistance of *Bacillus stearothermophilus* spores and concluded that the simultaneous application of moderate pressure (up to 30 MPa) and heat cannot be used as a preservation method, since the effect of pressure on the reduction of heat resistance is low at high temperatures. Nakayama et al. [85] observed no appreciable reductions in the spore viability for six *Bacillus* strains, including *B. stearothermophilus*, *B. coagulans*, *B. subtilis*, *B. licheniformis*, and *B. megaterium*, in pressure treatments at 5–10°C for 40 minutes up to pressures of 981 MPa or at 588 MPa for 120 minutes. Therefore, there is no possibility of pressure sterilization of low-acid foods at low temperatures. Hayakawa et al. [86] reported successful treatments for *B. stearothermophilus* spores when pressure treatments were combined with moderate temperature (70°C). Pressure treatments were four to six compression–decompression cycles at 600 MPa with 5 minutes of holding time. These treatments reduced by 6 to 7 log cycles the initial inocula. Lechowich [87] mentioned that there are no published reports on the

high-pressure resistance of *C. botulinum* spores, and their ability to support high pressure at low or high temperatures is unknown. There is a need for data concerning the high-pressure resistance of *Bacillus* and *Clostridium* spores. With this information, the safety aspects of high-hydrostatic-pressure treatment of low-acid foods can be documented [87].

Spores from yeast and molds are easily inactivated at pressures of 300 (*Aspergillus oryzae*) or 400 MPa (*Rhyzopus javanicus*) at ambient temperatures [18]. However, Butz et al. [88] demonstrated that ascospores of heat-resistant molds such as *Byssochlamys nivea* are extremely pressure resistant. For the inactivation of *B. nivea* ascospores, pressures above 600 MPa and temperatures above 60°C were needed. No effects on spore viability after treatment at 70°C and 500 MPa for 60 minutes were observed; a 3 log cycle reduction was observed at 600 MPa after 60 minutes; approximately 5 log cycles were reduced at 700 MPa; and total and rapid inactivation of *B. nivea* within a few minutes (<10) were observed at 800 MPa.

Bacterial spores represent a challenge for high-pressure technology, and more information about their resistance is required. Data are needed on the destruction of *C. botulinum* spores and the most resistant spore-forming species, and studies of the high-pressure process need to include inoculation and challenge testing.

49.4.3 Kinetics of Microbial Inactivation

The patterns of high-hydrostatic-pressure inactivation kinetics observed with different microorganisms are quite variable. Some investigators indicate first-order kinetics in the case of several bacteria and yeast [64, 67, 68, 89]. Other authors observed a change in the slope and a two-phase inactivation phenomenon: the first fraction of the population being quickly inactivated, whereas the second fraction appears to be much more resistant [18]. The pattern of inactivation kinetics is also influenced by pressure, temperature, and composition of the medium [66]. For a broader use of high pressure in food processing, it is of special interest to determine the process conditions for pressure pasteurization in view of industrial applications [18]. To increase microbial safety and ensure microbial stability of foods processed by high pressure, the pressure treatment must ensure a satisfactory reduction in the initial microbial counts, thus kinetic analysis and the pressure dependence of microbial-inactivation rates are needed.

Several scientific reports [54, 60, 79] demonstrate the efficacy of high-hydrostatic-pressure treatments against different microbial species. However, few of them reported kinetic data, which would be necessary for product and process design. For low-acid foods in particular, the kinetic information for pathogenic bacteria and spores will be indispensable in terms of food safety. The kinetic nature of high-pressure inhibition and inactivation may be different from that detected in heat treatment and other food-processing methods. Deviations from first-order kinetics, occurrence of survivor "tails" in death kinetics, and the possibility of cell recovery after pressurization have been observed [52, 53].

Earnshaw [52] stated that there is clear evidence from his and other laboratories that pressure-mediating death is not first order, and inactivation curves often present pronounced survivor tails. Thus the *D* and *z* concepts commonly used in thermal processing cannot be usefully applied to describe pressure processes. However, there is a difference with heat-treatment kinetic evaluation and sampling, and therefore the survivor enumeration is not continuous. Determination of microbial pressure-inactivation kinetics depends on pressure increase and decrease rates since it includes a decompression step to perform sampling. The pressure increase and decrease are not always reported, and the come-up time to reach the pressure is therefore not always taken into account in the logarithmic representation of the number of survivors. Cheftel [18] also mentioned that some publications do not precisely and fully indicate the experimental variables such as pressure, temperature, and time conditions. Moreover, Earnshaw [52] notes that in experiments that evaluate high-pressure-inactivation kinetics, the starting population must always be given. Cheftel [18] suggested that only inoculation with high numbers of a specific strain permits precise determination of the extent of inactivation as a function of processing conditions.

In many reported survival curves, it is unclear if time-zero experiments are growth controls without pressure treatment or are pressure treatments that only take into account the come-up time to reach the pressure level. Figure 49.7 presents the come-up times for pressure treatments ranging from 241 to 517 MPa [90]; the time to release the pressure was less than 15 seconds. The pressure come-up times exert an important

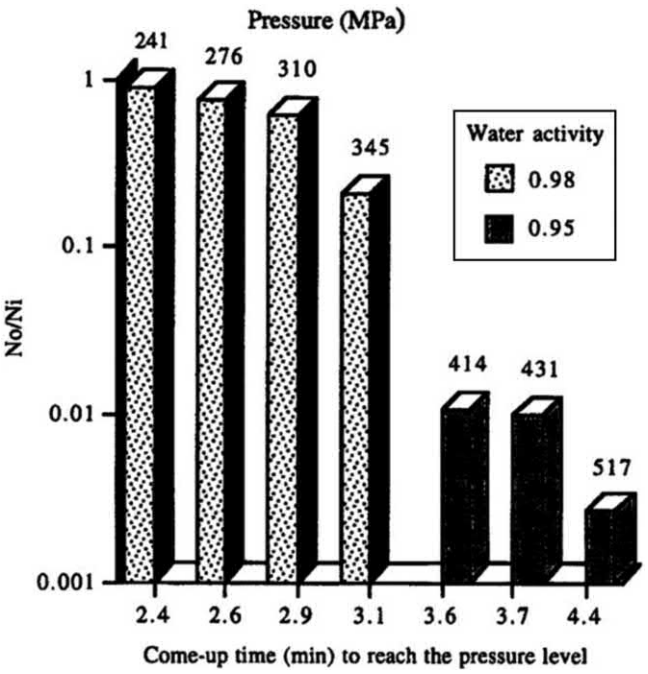

FIGURE 49.7 Effect of initial water activity (a_w), pressure, and pressure come-up time on *Zygosaccharomyces bailii* initial population reduction (N_o/N_i). N_o = yeast count (cfu/ml) after the come-up time for the working pressure; N_i = yeast initial population (cfu/ml). (From Palou et al. [90].)

effect on the yeast survival fraction. *Z. bailii* counts decreased as pressure increased, and at pressures greater than 414 MPa, 2 log cycle reductions were observed. Cheftel [18] reported that the rate of pressure increase and decrease is often neglected as an experimental variable in high-pressure microbial-inactivation studies, and the initial population (N_1) can be notably reduced during the come-up time (Figure 49.7). Palou et al. [90] reported an important effect of the come-up time at pressures of 345 and 517 MPa on *Z. bailii* log reductions in a food model system (a_w 0.98 and 0.95; pH 3.5), furthermore, total inhibition of the initially inoculated cells was achieved when only the time to reach 689 MPa was applied. Therefore, the effects of the rate of pressure increase and decrease need to be evaluated and reported.

Palou et al. [90] observed first-order kinetics for *Z. bailii* inoculated in food model systems with pH 3.5 and a_w 0.98 and 0.95. The logarithm of the survival fraction decreased linearly with time, and yeast cells were inactivated more rapidly with increasing pressure treatments (Figure 49.8). The experimental points with a pressure treatment duration of "0 minutes" express the effect of the come-up time to reach the working pressure and correspond to the initial population (N_o) for the kinetic analysis. Hashizume et al. [68] observed that pressure inactivation kinetics for *S. cerevisiae* at 25°C and a_w 0.99 follows a first-order kinetic model. The death velocity constants or inactivation rates (k) can be calculated from the reciprocal of the slope of the survival curves following a traditional kinetic analysis. Table 49.4 presents the velocity constants reported by Palou et al. [57] for *Z. bailii* and those calculated from the data reported by Hashizume et al. [68]. As can be seen, the k values suggest that *S. cerevisiae* is more pressure-resistant than *Z. bailii*. To compare the effectiveness of pressure treatments and to optimize process conditions, the calculation of D values can be used to compare the resistance

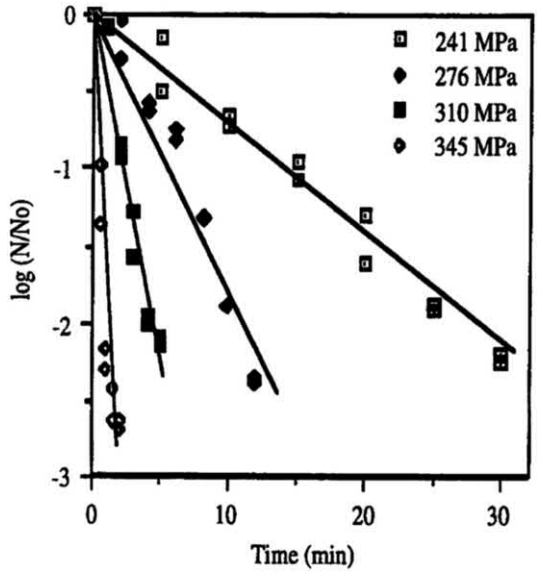

FIGURE 49.8 First-order pressure-inactivation kinetics of *Zygosaccharomyces bailii* in a laboratory model system with water activity 0.98 and pH 3.5. (Adapted from Palou et al. [90].)

TABLE 49.4
Effect of Initial Water Activity (a_w) and Pressure on Inactivation Rates (k) and Decimal Reduction Times (D) of *Zygosaccharomyces bailii* at 21°C and *Saccharomyces cerevisiae* at 25°C

a_w	Pressure (MPa)	k (min^{-1})	D (min)
Z. bailii[a]			
0.98	241	0.176	13.12
	276	0.478	4.82
	310	1.128	2.04
	345	2.833	0.81
0.95	414	0.902	2.55
	431	1.099	2.09
	517	2.645	0.87
S. cerevisiae[b]			
0.99	210	0.025	94.00
	240	0.067	34.63
	250	0.094	24.60
	270	0.187	12.30

[a] From Palou et al. [90].
[b] From Hashizume et al. [68].

of microorganisms. By analogy, with the analysis of thermal destruction or inactivation of microorganisms, a decimal reduction time (D value) can be defined as the time needed to reduce 90% the initial population and can be calculated as $D = 2.303/k$.

Calculated D values for *Z. bailii* [90] and *S. cerevisiae* [68] are presented in Table 49.4. Other reports detected first-order inactivation rates and decimal reduction times for bacterial inactivation with high hydrostatic pressure. Carlez et al. [64] observed that the number of surviving *Pseudomonas fluorescens*, *Citrobacter freundii*, and *Listeria innocua* inoculated in a minced meat product decreased exponentially with process time. Carlez et al. [64] reported decimal reduction times at 20°C of $D_{230\,MPa} = 14.7$ minutes for *C. freundii*, $D_{250\,MPa} = 23.8$ minutes for *P. fluorescens*, and $D_{230\,MPa} = 6.5$ minutes and $D_{360\,MPa} = 5$ minutes for *L. innocua*. Smelt and Rijke [67] reported first-order kinetics for high-pressure inactivation of *E. coli* in a physiological saline solution at 20°C, with D values of 25.9, 8.0, 2.5, and 0.8 minutes for treatments at 200, 250, 300, and 350 MPa, respectively. The pressure resistance of microorganisms depends on the type of microorganism and composition of the suspension media. Many factors that could affect the microbial response under high-pressure treatments, such as temperature, gas solubility, ionic strength, pH, and cavitation, are also modified by pressure. Thus, the microbial inactivation curves that can be obtained during a high hydrostatic pressure treatment also depend on these factors.

Figure 49.9 presents the fit of the experimental data obtained from Palou et al. [57] and Hashizume et al. [68] to Equation (49.1). The positive slope obtained from the plots of pressure inhibition rates (ln k) versus pressure, and therefore a negative activation volume, indicates that a decrease in

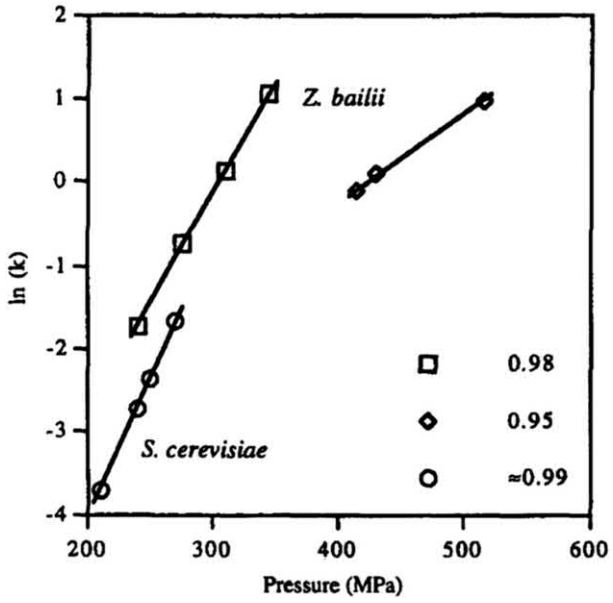

FIGURE 49.9 Effect of pressure and initial water activity on the inactivation rate (k) of *Zygosaccharomyces bailii* at 21°C and *Saccharomyces cerevisiae* at 25°C. (Adapted from Hashizume et al. [68].)

volume is related to the yeast-inhibition process. The activation volumes reported by Palou et al. [57] are presented in Table 49.5. The values obtained are quite different between system compositions and between yeast species. The apparent ΔE_v, calculated considering the activation process of microbial inhibition as one step, indicates the volume variation between the "activated complex" and initial states of the yeast pressure inhibition "reaction" [90]. A negative activation volume represents a reaction favored by increased pressure, so a reaction with a greater absolute ΔE_v value indicates that increments in pressure can accelerate the response, in this case the yeast inactivation rate.

A "pressure z value" can be defined as the pressure increment needed to reduce or increase the *D* value by a factor of 10 and is calculated as the reciprocal of the slope in a plot of log *D* versus pressure. Table 49.5 also presents z values for *Z. bailii* in food model systems at a_w 0.95 and 0.98 [90]. The z value for yeast inactivation in media with a_w 0.98 was 2.6 times smaller than that obtained for media with a_w 0.95, showing that *Z. bailii* was more pressure sensitive in the former condition. These results indicate that the composition of the media influences the microbial response under high-hydrostatic-pressure treatments and demonstrates the baroprotective effect of high sugar concentration or reduced a~ reported by several authors [49, 57, 68, 70, 73, 91]. The z value (68 MPa) calculated from the data reported by Hashizume et al. [68] indicates that *S. cerevisiae* is more sensitive to changes in pressure than *Z. bailii*, which can be attributed to response differences between microorganisms to high-pressure treatments and composition of the suspension medium.

In biological systems, the volume changes associated with ionization can be involved in the mechanism of microbial inactivation [57]. Enhanced ionization under high-pressure treatments is reported for water and acid molecules [52]. Palou et al. [57] mentioned that in the conditions of their work, and knowing that during pressurization a decrease in the pKa of the acids and pH reduction is expected, a temporary reduction in pH and an increase in the dissociated form of the acid can be present during pressurization. The pH changes could enhance the effects of high-pressure treatments on microorganisms and favor the first-order kinetics observed for pressure inactivation of *Z. bailii*.

In several process conditions and different microorganisms, the inactivation curves do not follow a first-order kinetics pattern [52, 53, 63, 79]. Figure 49.10 presents a comparison of high-pressure treatments at 20°C for *Yersinia enterocolitica* suspended in a pH 7.0 phosphate buffer [53]. Similar exponential decay curves have been reported for *E. coli*, *L. monocytogenes*, *S. typhimurium*, and *S. enteritidis* by the same authors at relatively high pressures. As can be seen for treatments at 275 and 300 MPa, the survival curves cannot be described by linear relations; various authors have proposed

TABLE 49.5
Activation Volume (ΔE_v) and z Values of *Zygosaccharomyces bailii* at 21°C and *Saccharomyces cerevisiae* at 25°C

a_w	Pressure Range (MPa)	ΔE_v (cm^3 mol^{-1})	z (MPa)
Z. bailii[a]			
0.98	241–345	–65.2	85.8
0.95	414–517	–25.3	222.7
S. cerevisiae[b]			
0.99	210–270	–83.9	68.0

[a] From Palou et al. [57].
[b] From Hashizume et al. [68].

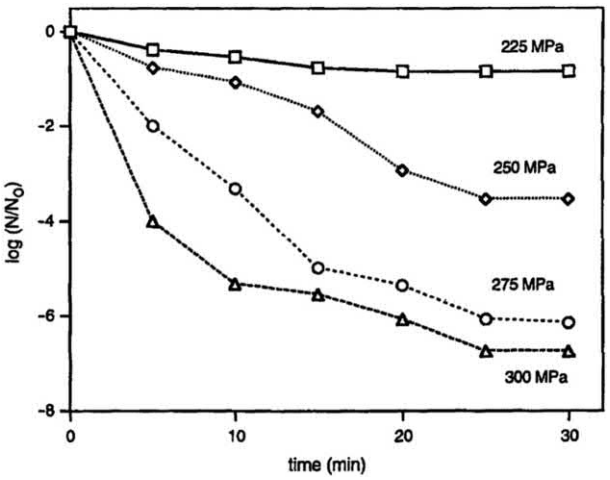

FIGURE 49.10 Effect of pressure on the inactivation of *Yersinia enterocolitica* in 10 mM buffered saline solution (pH 7.0) at 20°C. N = number of survivors; N_o = initial number of microorganisms. (Adapted from Patterson et al. [53].)

the logistic model to describe this pattern. However, the reliability of the logistic model for other organisms under different conditions and process variables needs to be investigated [92]. If the logistic model describes the experimental data, the time required to reduce the initial population by a factor of 10 (D value) can be calculated and used as a comparison parameter.

There are a number of possible theories to explain the tail effect [52]: tailing is a normal characteristic associated with the inactivation or resistance mechanisms, is independent of the mechanisms of survival and/or inactivation, and is the result of microbial population heterogeneity or is the result of experimental errors. Another possible reason for tailing is microbial adaptation and recovery during and after pressure treatment.

49.4.4 High-Pressure Mechanism of Action

In the inactivation of microorganisms by high pressure, the membrane is the most probable key site of disruption [53]. Inactivation of key enzymes, including those involved in DNA replication and transcription, is also mentioned as a possible inactivating mechanism [4]. The lethal effect of high pressure on vegetative microorganisms is thought to be the result of a number of possible changes taking place simultaneously in the microbial cell [53]. Shimada et al. [93] suggested that the structural impact of the high hydrostatic pressure on yeast cells occurred directly in the membrane system, particularly in the nuclear membrane. Besides membrane damage, a decrease in pH due to the enhancement of the ionic dissociation resulting from electrostriction (pressure causes the separation of electrical charges because the electrical charges "organize" water molecules around them, with a resulting decrease in the total volume of the system) during high-pressure treatments was reported by Cheftel [18]. Smelt [94] observed that the intracellular pH decreased under pressurization and associated the pH drop with the loss of ATPase activity and the reduction of the proton efflux from the cell interior. Knorr [16] reported that the reduced Na/K ATPase activity during and after pressurization can be related to a decrease in the bilayer membrane fluidity. Smelt [94] postulated that to maintain the internal pH homeostasis, membrane-bounded ATPase acts as an ion pump. High pressure can denature the enzyme or cause a dislocation in the membrane, thus microbial cells could die by internal acidification.

Microbial death is attributed to permeabilization of the cell membrane after a high-pressure treatment [3]. Pressure affects several biochemical reactions, and this kind of disturbance may be attributed to volume changes during compression, thus any biological process would be affected when high pressure is applied [4]. Farr [3] established that protein denaturalization can be attributed to changes in the chain conformational arrangement. Water pH is reduced from 7.00 (0.1 MPa and 25°C) to 6.27 when 101 MPa is applied [75], and water volume is also reduced as shown in Figure 49.5. These effects can also contribute to microbial inactivation by high pressure.

High-hydrostatic-pressure treatments can alter the membrane functionality such as active transport or passive permeability, and therefore perturb the physicochemical balance of the cell [95]. The physical state of the lipids that surround membrane proteins plays a crucial role in the activity of membrane-bound enzymes, and there is considerable evidence that pressure tends to loosen the contact between attached enzymes and membrane surfaces as a consequence of the changes in the physical state of lipids that control enzyme activity [20]. Jaenicke [96] reported that pressures in the range of 101–304 MPa denature several enzymes and treatments at 304 MPa make the phenomena irreversible. The activity of succinate, formate, and malate dehydrogenases in *E. coli* decreases with an increase in pressure. The dehydrogenases are completely inactivated when subjected to a pressure of 100 MPa for 15 minutes at 27°C [97]. Thus, the microbial inactivation mechanism by high pressure can be attributed, at least partially, to enzyme inactivation [4].

Perrier-Comet et al. [95] observed that yeast cell volume variations during a pressure treatment at 250 MPa for 15 minutes can be divided into three phases. A first phase of volume decrease occurs during the come-up time to reach the pressure; a second phase occurs during the holding time when the cell volume still decreases although pressure remains constant. The volume decrease is attributed to mass transfer between external and cellular media. A third phase of volume variation is attributed to membrane compression. The initial cell volume was not recovered during decompression or after returning to atmospheric pressure. An irreversible mass transfer (mainly water) occurs during the holding time of a pressure treatment.

High-pressure treatment also induces morphological changes in microbial cells. Separation of the cell wall and disruption in the homogeneity of the intermediate layer between the cell wall and the cytoplasmic membrane occur. Isaacs et al. [63] demonstrated with electron microscopy studies that ribosomal destruction in cells of *E. coli* and *L. monocytogenes* results in metabolic malfunctions that can cause cell death. Mackey et al. [98] also observed by electron microscopy that the nuclear material appearance changes considerably in *L. monocytogenes* and *Salmonella thompson* after being treated at 500 MPa for 10 minutes. Hayakawa et al. [86] reported morphological changes in the *B. stearothermophilus* spore surface after pressurization for six cycles of 5 minutes each under 600 MPa at 70°C and observed that every spore was completely ruptured after this process. These observations were attributed to a weakening of the physical strength of the spore coat and rupture of the coat as a result of the pulsed pressure treatment. For *Schizosaccharomyces pombe*, after a treatment at 100 MPa the nuclear membrane was damaged and fragmented [99]. In the same study, a pressure treatment above 250 MPa dramatically changed the cytoplasmic substance, the cellular organelles could hardly be detected, and the fragmented nuclear membrane was barely visible. The outer cell shape, observed by scanning electron microscopy (SEM), of *S. cerevisiae* was almost unaffected by high-pressure treatments up to 300 MPa, but at pressures higher

than 500 MPa there was disruption and damage to the cell wall [93]. Transmission electron microscopy (TEM) revealed that the inner structures were damaged, especially the nuclear membrane, even at 100 MPa [93]. The damage profile of high-pressure treatments revealed that *S. pombe* cells were more affected at low pressure stresses than were *S. cerevisiae* cells [99].

Isaacs et al. [63] observed that pressure treatments at 200–400 MPa cause leakage of ultraviolet (UV)-absorbing materials from *E. coli* cells. Shimada et al. [93] reported that leakage of intracellular UV -absorbing substances from *S. cerevisiae* began to be released at relatively low pressures (100 MPa). When pressure was 200 MPa for 10 minutes, the leakage gradually increased. Leakage of internal substances was related to cell viability, and at 300 MPa most of the *S. cerevisiae* cells were inactivated, corresponding to the great concentration of UV-absorbing substances. This can be attributed to increased permeability and fluidity, and might also provide evidence about the mechanism of high-pressure inactivation [100].

To explain the response of microorganisms to different pressures, high-pressure effects on several biological molecules have been studied; protein denaturation, lipid phase change, and enzyme inactivation can perturb the cell morphology, genetic mechanisms, and biochemical reactions. However, the mechanisms that damage the cells are still not fully understood [95].

49.5 EFFECTS ON ENZYMES

Exposure to high pressure may activate or inactivate enzymes. Pressure inactivation of enzymes is influenced by pH, substrate concentration, the subunit structure of the enzyme, and temperature during pressurization [4]. Pressure effects on enzyme activity are expected to occur with the substrate–enzyme interaction. If the substrate is a macromolecule, then the effects may be on the conformation of the macromolecule, which can make the enzymatic action easier or more difficult [20]. Pressure enzyme inactivation can also be attributed to an alteration of intermolecular structures or conformational changes at the active site. Inactivation of some enzymes pressurized to 100–300 MPa is reversible. Reactivation after decompression depends on the degree of distortion of the molecule. The chances of reactivation decrease with an increase in pressure beyond 300 MPa [96, 101].

Earnshaw [8] mentioned that of particular significance is the apparent lack of pressure effect on some food enzymes, including those that affect food quality, such as proteases, lipases, esterases, and oxidases. Some enzymes, such as phosphatase, are relatively pressure-sensitive and can be inactivated by pressures in the range of 400–800 MPa. The enzymes alkaline phosphatase and lactoperoxidase have been successfully used as process markers in milk heat treatments. Quality control markers such as these enzymes will be needed for high-pressure processing of dairy products. Figure 49.11 presents the effect of 20-minute pressure treatments on alkaline phosphatase in raw milk; the enzyme activity decreases

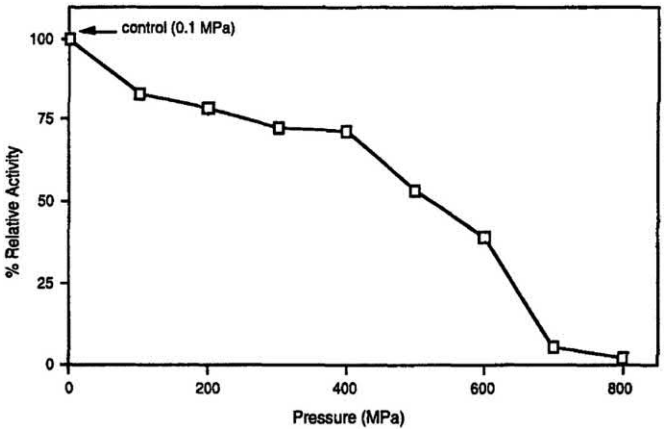

FIGURE 49.11 Relative activity of enzyme alkaline phosphatase in raw milk after 20 minutes of pressure treatment. (Adapted from Johnston [117].)

as the pressure level increases. However, there is a need to establish microbial-quality marker relations to ensure, for example, the destruction of *Mycobacterium tuberculosis* [24].

Heremans and Heremans [102] reported that chymotrypsin is an enzyme that exhibits a pressure-induced conformational change. Its optimal activity is at neutral pH, and the activity disappears at pH 10, which is attributed to rupture of the salt bridge in the vicinity of the active site. Raman spectroscopy studies indicated that pressure inactivates the enzyme due to the destabilizing effects of pressure on the salt bridge and revealed that below 400 MPa the conformational change is reversible [102].

Homma et al. [103] mentioned that high hydrostatic pressure is one of the new technologies that can be used for tenderizing meat or accelerating meat conditioning. Pressure induces changes in the muscle that could be derived from the physical force and/or increase in the proteolytic activity of meat enzymes. The proteolytic activity of enzymes in meat is enhanced by the application of high pressures [103]. The total activities of cathepsin B, D, L, and acid phosphatase in the muscle increase when subjected to pressures ranging between 100 and 500 MPa for 5 minutes at 2°C. Cathepsin H and aminopeptidase B are resistant to pressure treatment. An increase in the activity of cathepsin B1 may account, in part, for the tenderization of meat by pressure-heat treatment [104]. Ashie et al. [105] reported a reduction in the proteolytic activity of fish muscle enzymes with increasing pressure in the range of 100–300 MPa (30 mm) and increasing crude inhibitor (α_2-macroglobulin) concentration (0.1–0.3%) at constant pH (5.5, 6.0, or 6.5). The combination of treatments could enhance the texture of fish muscle, since it favors the inactivation of proteolytic enzymes. This kind of result could have widespread use in surimi and other minced fish products, which have the problem of undesirable protease activity resulting in gel softening.

Enzymes are generally inactivated in vegetables by hot water blanching. Disadvantages of blanching include thermal damage, leaching of nutrients, and possible environmental

pollution due to the production of high biochemical oxygen demand effluent. High-pressure treatment can fulfill the requirements of hot water blanching while avoiding mineral leaching and accumulation of wastewater. High-pressure treatment produces less effluent because less water is required than in hot water blanching [106]. Quaglia et al. [107] reported that pressure treatment at 900 MPa for 10 minutes reduces the peroxidase activity 88% in green peas comparable to traditional water blanching. However, the pressurization treatment resulted in greater ascorbic acid and firmness retention. Lower pressure levels decreased the enzyme activity less than 50%, even when pressure was combined with moderate temperatures (39–60°C). Anese et al. [108] also observed in peroxidase from a carrot cell-free extract that a complete loss of enzyme activity was achieved only when the pressure treatment was applied at 900 MPa for 1 minute. Enzyme activation was observed for treatments in the range of 300–500 MPa. For polyphenoloxidase (apple cell-free extract) it was observed that at pH 7.0, 5.4, and 4.5 a significant reduction in enzyme activity occurred in pressure treatments at 900 MPa for 1 minute. For both enzymes a pH dependence on residual activity after the pressure treatment was observed. Eshtiaghi and Knorr [106] reported that the addition of citric acid could lead to increased polyphenoloxidase inactivation since pH reduction enhances the pressure effects on enzyme inactivation. Denaturation and inactivation of enzymes occur only when very high-pressure treatments are applied; the activation effects that could be presented at relatively low pressures could be attributed to reversible configuration and/or conformation changes on enzyme and/or substrate molecules [108, 109]. Seyderheim et al. [110] evaluated the effects of high-hydrostatic-pressure treatments on selected enzymes including catalase, phosphatase, lipase, pectinesterase, lypoxygenase, peroxidase, polyphenoloxidase, and lactoperoxidase, and reported that peroxidase was the most barostable enzyme with 90% residual activity after 30-minute treatment at 60°C and 600 MPa. Therefore, peroxidase could be used as an enzyme indicator for high-pressure treatments.

Pressurization at 100 and 200 MPa causes hardly any inactivation of pectinesterase [70]. Pectinesterase in juices such as Satsuma mandarin juice is inactivated when pressurized to 300–400 MPa. Purified pectinesterase is also inactivated at pressures of 300 MPa or higher. The inactivation is irreversible, and the pectinesterase is not reactivated during storage at 0°C or transportation. The activity of pectinesterase from mandarin juice remains at low levels during 90 days of storage at 0°C after pressure treatments at 400–600 MPa. Soluble solids such as sugars, proteins, and lipids exert a protective action against pectinesterase inactivation by high pressure or heat [70]. Polyphenoloxidase is often described as a soluble enzyme, localized mainly in the cytosol of plant cells, and is associated with particulate cell fractions [111]. It is well established that polyphenoloxidases from different sources may have different molecular sizes and conformations. Thus, it is expected that the polyphenoloxidases may respond differently during and following high-pressure treatments. It is also anticipated that important differences will occur when

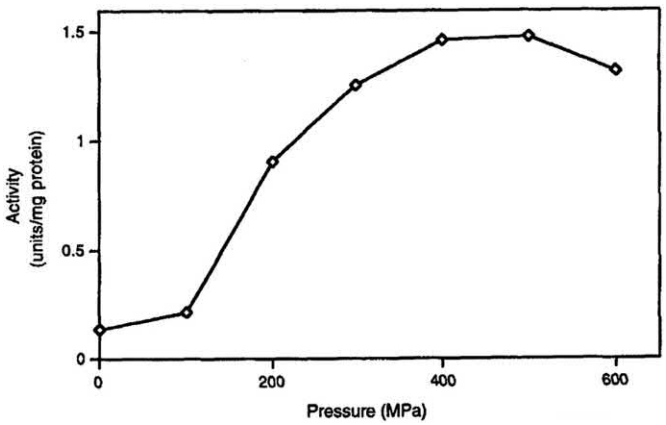

FIGURE 49.12 Activation of polyphenoloxidase from Bartlett pears by high pressure. (Adapted from Asaka and Hayashi [112].)

the enzyme activity is analyzed in whole foods, extracts, or commercial enzymes. In untreated onion cells, phenolic compounds are confined to vacuoles and spatially separated from the polyphenoloxidase by the tonoplast; after pressurization (>100 MPa) the cell and the tonoplast are disrupted and phenolic oxidation products are formed. Polyphenoloxidase is no longer separated from the substrate, and enzymatic browning begins [111]. The activity of polyphenoloxidase increases five times when slices of Bartlett pears are pressurized at 400 MPa and 25°C for 10 minutes (Figure 49.12). A further increase in pressure does not increase enzyme activity. On the other hand, pressurization of homogenates of apples, bananas, or sweet potatoes did not result in the activation of polyphenoloxidase [112]. Gomes and Ledward [113] reported a reduction in polyphenoloxidase activity from a crude potato extract with increasing pressure (400–800 MPa for 10 mm). In contrast, when the crude extract of mushroom was treated at 400 MPa for 10 minutes, an enhancement in the activity was observed. Pressures above 300 MPa inactivated polyphenoloxidase in apple slices [114]. Cano et al. [115] studied the combination of high pressure and temperature on peroxidase, polyphenoloxidase, and pectin methylesterase activities of fruit-derived products. Optimal inactivation of peroxidase in strawberry puree was achieved using 230 MPa and 43°C. Pressurization–depressurization treatments caused a significant loss of strawberry polyphenoloxidase up to 230 MPa. Combinations of high pressure and 35°C effectively reduced peroxidase in orange juice. The effects of pressure and temperature on pectin methylesterase activity in orange juice were similar to those for peroxidase. There is some evidence of changes in the enzyme–substrate interactions during pressurization and, therefore, changes in enzyme reaction kinetics. Some beneficial aspects of enzyme activation or reduced enzyme activity by high pressure can be used to retain or increase food quality.

49.6 CHEMICAL REACTIONS AT HIGH PRESSURE

The application of pressure influences biochemical reactions since most of these reactions involve a change in volume.

Hoover et al. [4] reported that pressure affects reaction systems in two apparent ways: by reducing the available molecular space and by increasing interchain reactions. Thus, reactions involved with the formation of hydrogen bonds are favored by high pressure since bonding results in a decrease in volume [4]. However, Masson [116] reported that hydrogen bonds are insensitive to pressure. Cheftel [18] mentioned that various biochemical studies indicate that pressures above 100–200 MPa often cause (i) the dissociation of oligomeric structures into their subunits; (ii) partial unfolding and denaturation of monomeric structures; (iii) protein aggregation, probably as a consequence of unfolding; and (iv) protein gelation if protein concentration and pressure are high enough.

The functional properties of biological molecules are usually dependent on conformation and conformational changes. The interactions between solvent and solute molecules and inter- and intramolecular interactions of the solute are influenced when subjected to pressure. Therefore, either beneficial or detrimental changes can be produced as a result of a high-pressure treatment [117]. Hydrogen bonding, which stabilizes protein structures (α-helix and β-pleated sheets), is influenced by pressure, but to a lesser extent than ionic or hydrophobic interactions. Hydrogen bond formation results in the shortening of interatomic distances with the corresponding volume decrease and is, therefore, enhanced by high pressure [117].

49.6.1 Structural Change in Protein

High pressure can denature protein molecules. Pressure denaturation of proteins is a complex phenomenon depending on the protein structure, pressure range, temperature, pH, and solvent composition. Oligomeric proteins are dissociated by relatively low pressures (200 MPa), whereas single-chain protein denaturation occurs at pressures greater than 300 MPa. Pressure-induced denaturation is sometimes reversible, but renaturation after pressure release may take a long time. Protein denaturation becomes irreversible beyond a given pressure threshold, which depends on the protein, or at high protein concentrations that enhance aggregation [18]. Figure 49.13 presents a schematic diagram illustrating the effect of temperature on the denaturation of proteins. This kind of diagram delimits regions where the protein is active or denatured; at high temperature, pressure stabilizes the protein against temperature denaturation [18, 20]. Heremans [20] mentioned that the fact that one can "cook" an egg with pressure is the result of the unique phase diagram of proteins. There is evidence that a similar phenomenon occurs with other biomolecules such as polysaccharides [20, 118], microorganisms and bacteriophages [119], and phospholipids [20].

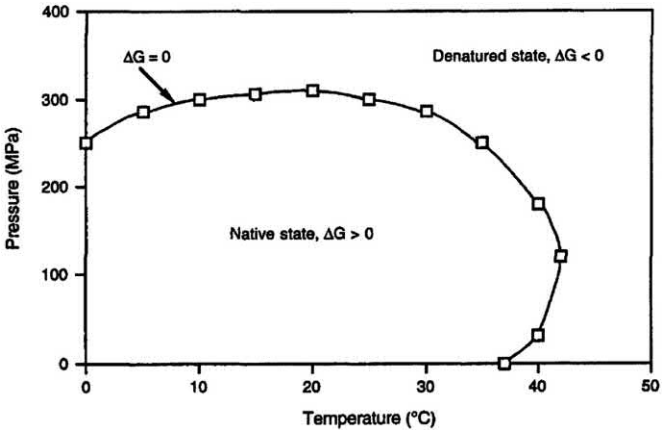

FIGURE 49.13 Schematic diagram illustrating the effect of temperature and pressure on the denaturation of proteins. (Adapted from Heremans [20].)

Pressure may affect the secondary, tertiary, and quaternary structure of proteins. The main targets of pressure are the electrostatic and hydrophobic bonds in protein molecules. High pressure causes deprotonation of charged groups and disruption of salt bridges and hydrophobic bonds, thereby resulting in conformational and structural changes of proteins. Structural transitions are accompanied by large hydration changes. Hydration changes are the major source of volume decreases associated with dissociation and unfolding of proteins [116]. Hydrophobic interactions in proteins can be either disrupted or stabilized according to the magnitude of the applied pressure [24]. The disruptive effect of high hydrostatic pressure on the ionic and hydrophobic interactions and hydrogen bonds of milk casein micelles would allow independent movement of micelle fragments along with caseins and calcium phosphate [24] and cause a conformational change in proteins. In fresh milk, casein micelles are large complex structures consisting of many molecules of different individual caseins and calcium phosphate, maintaining structural integrity. The structural and spatial distribution changes to the micelle components during pressurization would prevent recovery of the micelle original structures after high-pressure treatment [24].

Exposure to high pressure unfolds protein molecules. Unfolding results in alterations of the functional properties of the protein [24]. Foaming, emulsifying, gelling, and water-binding capacity of proteins may be influenced. Proteins treated with high pressures may lead to the development of a range of functional food ingredients prepared from food proteins by controlled unfolding [24]. It is well known that pH and ionic strength influence protein aggregation and gelation under heating conditions; this is also observed when protein solutions are subjected to high hydrostatic pressure. Cheftel [18] mentioned that it is likely, yet not fully demonstrated, that protein aggregation and gelation occur under pressurization as well as after pressure release.

Yogurts (without addition of milk powder) are improved by HP with a high-water retention capacity and without syneresis during storage, and an improved texture as compared to conventionally processed yoghurt. The microstructure and texture of cheeses are improved by retaining whey with higher protein interactions, and partial disruption or aggregation of casein micelles [120].

Low-protein wheat flour does not positively affect the quality of final products of noodles and bread. Lee and Koo [121] observed that high hydrostatic pressure (150 MPa) improved the physicochemical characteristics (i.e., water holding

capacity, and viscosities) of blended low-protein wheat and oat flour, and improved quality (i.e. hardness, gumminess and chewiness) of produced noodles.

49.6.2 Structure in Lipids

Other food components that can be affected by high-pressure treatments are lipids. Ohshima et al. [122] observed that in cod muscles exposed to high-pressure treatments in the range of 202 to 608 MPa for 15 and 30 minutes, the peroxide value of the extracted oils increased with increasing pressure and processing time. The presence of fish muscle accelerates the lipid oxidation after high-pressure treatments, while the isolated oil extract was relatively stable against autoxidation in treatments up to 608 MPa [123]. Cheah and Ledward [124] observed the effects of high-pressure treatments on lipid oxidation in rendered pork fat. Pork samples treated at 800 MPa for 20 minutes and stored at 50°C presented a shorter induction time for lipid oxidation and greater peroxide and TBA (2-thio-barbituric acid) values than untreated pork samples. When pork was stored at 25°C the induction periods were longer than at 50°C, and at 4°C after 8 months of storage the peroxide value was higher than in the untreated samples.

The effects of a_w were also evaluated by Cheah and Ledward [124], who reported that after 4, 6, and 8 days of storage at 50°C, the lipid oxidation in terms of peroxide and TBA values of high-pressure-treated (800 MPa, 20 mm) pork fat was inhibited to some extent at a_w values higher or lower than 0.44. In another study, Cheah and Ledward [125] reported that pressure treatments had little effect on lipid oxidation of minced pork, in terms of TBA value, below 300 MPa but increased proportionally at higher pressures. Pressure treatments in the range of 300–400 MPa appear to be critical for inducing marked changes in pork meat; many structural changes are induced at these pressures. These results may restrict the application of high-pressure technology for meat-based products due to induced oxidation, at least in the mentioned pressure range.

Fat crystallization by high-hydrostatic-pressure treatments is another interesting aspect to be considered. Buchheim and Abou El-Nour [126] observed that fat crystallization increased with the extent of the pressure treatment; maximum changes were found in the pressure range of 300–350 MPa. Dumay et al. [127] stated that this behavior can be used for aging ice cream mixes and physical ripening of dairy cream in butter making. These can be considered as potential applications of high-pressure technology.

49.6.3 Maillard Reaction

The application of high-hydrostatic-pressure treatments, in combination with moderate or elevated temperatures, may influence chemical reactions inherent to food systems such as the Maillard reaction. This reaction, which is manifested by the development of brown color in many processed foods, is known to be highly pH and temperature-dependent. However, few studies have dealt with the effects of high pressure on the Maillard reaction. Tamaoka et al. [128] reported that brown color development was inhibited at pressures in the range of 200–400 MPa in xylose-lysine systems (pH 8.2) when heated at 50°C. They also pointed out that Maillard reactions involving xylose-lysine, xylose-β-alanine, or glutaraldehyde β-alanine are inhibited by high-pressure treatments. Hill et al. [129] compared the rate of browning of glucose-lysine systems at 50°C, in the pH range of 5.1–10.1, with and without the application of high pressure at 600 MPa. Hill et al. [129] reported that at initial pH of 8.0 or 10.1, pressure enhances browning, while at pH 6.5 and 5.1 the effect is the opposite. At 600 MPa the rate of browning was significantly reduced. These observations were attributed to the pH decrease in the systems during pressurization. At pH 6.5 and 5.1 the system buffer capacity is due to the carboxylic acid group of the amino acid, and decreases of about 1.2 pH units occurred. Hill et al. [129] also demonstrated by high-performance liquid chromatography (HPLC) and UV spectra that the composition of the reaction products is similar in samples with the same intensity of browning, whether the samples were treated with high pressure or not. Acid hydrolysis of proteins is enhanced under high-pressure treatment, whereas hydrolysis of corn starch and locust bean gum is unaffected [12, 130]. Pressure treatments at 392–490 MPa at temperatures of 45–50°C enhanced the susceptibility of wheat, corn, and potato starches tα-amylase action [30].

49.6.4 Gelation and Gelatinization Processes

Phase changes in proteins and lipids are accompanied by the application of high pressure; these modifications offer opportunities to develop new products with unique rheological properties [8]. The structure of food proteins and polysaccharides can be changed by high-hydrostatic-pressure treatments and confer different rheological properties and mouthfeel. Earnshaw [8] explained that some Japanese researchers claim that these changes are desirable and that the gel quality of surimi can be improved with high-pressure treatments.

The process of gel formation is the macroscopic consequence of the denaturation, on a molecular level, of proteins and other biomacromolecules such as polysaccharides. The denatured state forms a gel or a precipitate, depending on the physical and chemical environmental characteristics.

Egg yolk subjected to a pressure of 400 MPa for 30 minutes at 25°C forms a gel. While a pressure of 500 MPa renders egg white partially coagulated and opaque, a pressure of 600 MPa causes complete gelation. Pressure-induced gels of egg white possess a natural flavor, displaying no destruction of vitamins and amino acids, and are more easily digested when compared to heat-induced gels. The gels retain the original color of the yolk or the white and are soft, lustrous, and adhesive when compared to heat-induced gels. While the strength of the gels increases, the adhesiveness decreases with an increase in the applied pressure. However, the hardest gel formed by high-pressure (500 MPa) treatment exhibits one-sixth the strength of heat-induced gels. Gumminess of pressure-induced gels is considerably less than gumminess

of heat-induced gels. Gels of egg white produced at 600 and 700 MPa deform readily without fracture. Cohesiveness of pressure-induced gels increases with increases in applied pressure. The force deformation curves of pressure and heat-induced gels of egg yolk and egg white are presented in Figure 49.14 [131]. Ibarz et al. [132] studied the viscoelastic characteristics of egg gels formed under several high-hydrostatic-pressure conditions. For egg yolk samples, gels were formed at pressures above 500 MPa, while for whole egg and egg white samples, gels were formed at pressures over 600 MPa. During amplitude sweep and frequency sweep tests storage modulus ′G and loss modulus "G of gels increased when the treatment pressure increased. ′G was greater than "G in every case studied. ′G values for boiled egg gels were greater than for pressurized gels.

In Japan, a hydrostatic pressure of 400 MPa is used to induce gelation of pollack, sardine, skipjack, and tuna-based surimi. Squid-based surimi is obtained by pressurization of extracted muscle protein at 600 MPa. Pressure-induced surimi gels are organoleptically superior to heat-induced surimi gels [3]. Gelation can be used for adhesion-binding of small size muscles or fish fillets, restructuring of minced fish or deboned meat, and molding of surimi or pieces of gelled surimi into seafood analogs. The possibility of obtaining acceptable gels simultaneously with commercial sterilization at a temperature as low as 0°C is of tremendous practical interest to the surimi industry [34].

The mechanism of high-pressure-induced gelation is different from heat-induced gelation. Gelation by high-pressure treatment is attributed to a decrease in the volume of the protein solution. On the other hand, the application of heat results in violent movement of protein molecules leading to the destruction of noncovalent bonds, denaturation, and formation of a random network. The rearrangement of water molecules around amino acid residues in pressure-induced gels produces glossy and transparent gels compared to opaque gels obtained by high temperatures [131].

Heremans [20] mentioned that important differences in the mechanical properties for temperature- and pressure-induced gels are expected. Hayashi [40] reported that pressure-coagulated food proteins, i.e., egg, soy protein, beef, pork, and fish meat, are more glossy, transparent, dense, smooth, and soft compared to boiled ones. These unique textural properties obtained by the pressurization offer ways to create new food materials.

Gelatinization is the transition of starch granules from the birefringent crystalline state to a nonbirefringent, swollen state. Starch can be gelatinized using pressure or heat. The pressure at which starch gelatinizes depends on the source of starch. Gelatinization may be stimulated by increased temperatures of pressurization [133]. High-pressure may also produce an upward shift of gelatinization temperature of about 3–5°C per 100 MPa. Pressures higher than 150 MPa do not further enhance the gelatinization temperature. The effect of high pressure on gelatinization is due to the stabilization of hydrogen bonds, which maintains the starch granule in the original state [118]. High-pressure treatments on starches produce unique properties, which are different from those formed by heat gelatinization. Pressure-treated starches keep the granular structure intact, whereas heat treatment destroys starch granules and dissolves the starches to give transparent solutions [40].

49.6.5 Vitamin C and Bioactive Compounds Degradation

Su et al. [134] observed that the degradation of anthocyanin and visual color of bayberry juice increased with the increase of HP treatment (pressure: 400, 500, 600 MPa; temperature: 25°C; time: 5–10 min). When stored at 4°C, degradation of untreated and HP treated juice showed similar trends, whereas higher degradation was observed in the case of untreated juice when stored at 25°C. The activation energies (E_v) for anthocyanin and color were observed as –19.85 and –14.92 cm^3 $mole^{-1}$, respectively, and this indicated that anthocyanin was more pressure sensitive than the visual color parameter [134].

HP treatment can increase the extraction capacity of phenolic constituents during juice processing and generally their stability as compared to the untreated is enhanced during storage [135]. The increase in yield is due to enhanced release, mass transfer rates, increased cell permeability, and diffusion of phenolic compounds [136]. The bioactive compound extractability in onion (i.e., increased phenolic and antioxidant and no effects on vitamin C, 100–600 MPa, 1–3 min, 25°C) [136]; carrot (residual PPO 6.9–15.1%) and spinach (residual PPO 21.3–31.1%) (i.e., increased flavonoid and antioxidant) [137]; and strawberry, wild

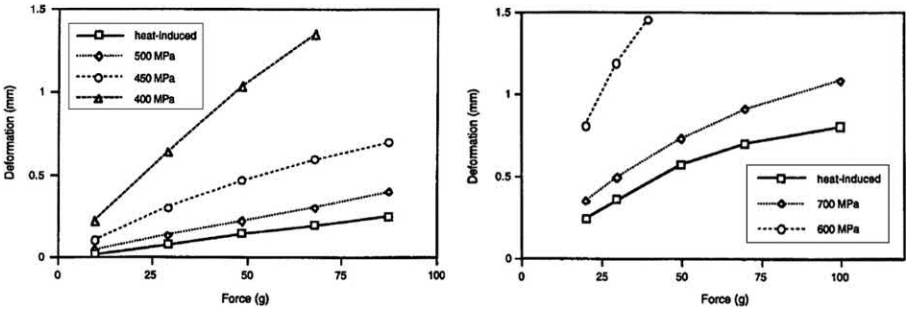

FIGURE 49.14 Force deformation curves of pressure- and heat-induced gels of egg yolk (left) and egg white (right). (Adapted from Okamoto et al. [131].)

strawberry, and pomegranate juice (i.e., polyphenol, anthocyanin and color pigments, 400–500 MPa, 25–50°C, 5–10 min) [138] are enhanced by HP processing. Purple sweet potato nectar treated with HP for a short time (400–600 MPa, 2.5–10.0 min) showed stability of total phenolic, anthocyanin, antioxidant, and color during storage. However, longer shelf life was observed when stored at 4°C as compared to 25°C [139].

HP processing better retained ascorbic acid and carotenoids in carrots and spinach (treated at 100, 300, and 500 MPa for 20 min) as compared to the thermal processing [137]. HP treatment caused a decrease of 10–15% of the initial vitamin C in the case of green pepper, while it increased 10–15% in the case of red peppers (100–200 MPa for 10–20 min) [140]. Similarly, stability of sulforaphane and phenylethyl isothiocyanate in broccoli juice increase in broccoli juice (600–880 MPa, 30–60°C) and these are thermo-labile and pressure stable [141].

49.6.6 Sensory Properties

The principal advantage of high-pressure technology is its relatively small effect on food composition and, hence, on sensory and nutritional attributes. Generally, pressure has little effect on food nutritional characteristics. However, more research is needed before solid judgments can be made [8]. Aleman et al. [142] studied the effect of high hydrostatic pressure on the natural flora present in fresh-cut pineapple. Treatments of 340 MPa for 15 minutes at –4°C, 21°C, or 38°C considerably reduced the initial counts of mesophilic bacteria, yeast, and molds, suggesting an increased shelf life in comparison with untreated pineapple samples. Alteration of the structure of starch and protein by high pressure can be utilized so that rice can be cooked in a few minutes [4]. Grapefruit juice manufactured by high-pressure technology does not possess the bitter taste of limonene present in conventional thermal-processed grapefruit juice [143]. Peaches and pears processed at 410 MPa for 30 minutes remained commercially sterile for 5 years [4]. Pressure treatment of nonpasteurized citrus juices provides a fresh-like flavor with no loss of vitamin C and a shelf life of approximately 17 months [3]. The internal structure of tomatoes becomes tough, tissues of chicken and fish fillets become opaque, and prerigor beef is tenderized [4]. Pressure has an influence on meat ultrastructure with similar changes to those observed during the age-conditioning of meat, therefore the juiciness and tenderness are affected [144].

The jams obtained by high-pressure processing retain the taste and color of fresh fruit, unlike the conventional jams produced by heat. In Japan, high-pressure processing is utilized for the manufacture of jams, marmalades, and sauces from strawberry, orange, and other fruits. The desired plastic container is filled with a mixture of raw materials consisting of fruits, fruit juice, sugar, and acidulants. The container is sealed and subjected to a pressure of 400–600 MPa for 1–30 minutes. Strawberry jam can be obtained by pressurization at 400 MPa for 15 minutes and strawberry puree by pressurization at 400 MPa for 10 minutes. Pressurization allows the permeation of sugar solution into the fruits as well as commercial preservation of the jam [145].

El Moueffak et al. [146] reported that duck foie gras pressurized at 50°C and 400 MPa for 10–30 minutes or at 300 MPa for 30 minutes presents attractive sensory characteristics, with less fat melting, softer texture, less cooked flavor, and a microbiological quality close to that obtained with traditional heat treatment. Pressure treatments up to 150 MPa did not change the sensory and instrumental color of minced beef muscle [65]. However, treatments for 10 minutes at pressures higher than 350 MPa turned the meat surface to a grayish tone, which corresponds to a decrease in the instrumental color a* value. The grayish tone was even more noticeable when 450 MPa was applied for 10 minutes.

Eshtiaghi et al. [147] observed that the color of dried green beans, carrots, and potatoes dried without pretreatment was dark due to enzymatic browning, while pressure or water-blanched pretreated vegetables retained an acceptable color. The pretreatments applied were water blanching in boiling water (carrot and green beans for 7 min and potatoes for 4 min) and pressure treatment at 600 MPa for 15 minutes at 70°C. The texture of dried and rehydrated pressure-pretreated green beans, carrots, and potatoes resulted in textures near that of raw vegetables.

Butz et al. [111] reported that diced onions subjected to high-hydrostatic-pressure treatments lose their typical pungency and characteristic odor due to an intense decrease in dipropyldisulfide content and an increase in 2-methyl-pent-2-enal. Dipropyldisulfide is the compound associated with the odor of fresh onion. Diced onions presented no major changes in appearance immediately after a 30-minute pressure treatment at 300 MPa and 25°C [111]. However, a slight glassy appearance, typical of steamed onions, was observed. In the same study, onions treated at 350 MPa and stored for 24 hours at 20°C exhibited an intense brown color. Onions treated at 300 MPa started to brown, whereas samples pressurized at 100 MPa remained unchanged. Any pressure treatment above 100 MPa induces browning of diced onions, and the rate of browning increases with increasing pressure. Microscopic evaluation of high-pressure-treated (300 MPa) onions revealed severe damage in the vacuoles of the epidermis cells, with the liberation of substrates for polyphenoloxidase activity [111].

Takahashi et al. [148] evaluated the effect of high-hydrostatic-pressure treatments on Satsuma mandarin juice and reported that juice pressurized up to 600 MPa for 5 or 10 minutes at 20–22°C did not change in chemical composition including soluble solids, acidity, amino nitrogen, vitamin C, and essential oil contents. The pressure-treated juices presented no off-flavor, and dimethyl sulfide, the characteristic compound found in off-flavor juices, was not detected. High-pressure-treated juices had very high scores in the sensory evaluation. Hayashi [40] reported some characteristics of food proteins treated by high-pressure technology: (i) Beef muscle pressurized at 400 MPa for 10 minutes looked like raw ham and the taste of pressurized beef was intact, even when the surface seemed slightly baked. (ii) In shrimp treated at 400 MPa for 10 minutes, no apparent changes in color or shape were observed. However, shrimp meat was coagulated as in boiled shrimp.

There are few studies regarding high-pressure effects on nutritional characteristics of pressure-treated foods. Elgasim and Kennick [149] reported that a pressure treatment at 103 MPa for 2 minutes improved the apparent digestibility of meat protein and had no adverse effect on the apparent biological value, net protein utilization, or protein efficiency ratio. A wide variety of effects and changes in food flavor, texture, physical appearance, and structure could result after the application of pressure, and these changes will depend on the type of food and its composition and structure.

Plant structures containing entrapped air will be affected by high pressure when the air volume is compressed. However, in some cases, the vacuoles and pores can be filled with the surrounding fluid, after which the material can maintain its structural integrity with increased density [8]. Adiabatic heating occurs in most food materials subjected to high pressure, and this is proportional to the compressibility of the food:air ratio. Entrapped air in the food matrix and cell vacuoles is very compressible and will increase the food or system temperature [8]. Some vegetable structures are resistant to pressure, while others can exhibit significant softening and severe color changes after pressurization. The effect of high-pressure treatments will depend on the kind of vegetable or fruit, physical characteristics, and maturity. Dumay et al. [127] evaluated the effects of high-hydrostatic-pressure treatments on the physicochemical characteristics of dairy creams and oil–water model system emulsions. Pressure treatments at 450 MPa did not affect the structure of the emulsions. Coalescence or emulsion breakdown was not observed. In the case of model emulsions, important rheological changes were observed, depending on the surface-active protein present and the effects of high pressure on unfolding and/or aggregation. Emulsions containing sodium casinate remained unchanged after treatment, while in those containin β-lactoglobulin the pressure treatment induced changes in their rheological behavior.

49.6.7 Volatile Formation

Chemical reactions in HP processing cause formation of different volatiles as compared to the thermal processing. In the case of carrot, Kebede et al. [150] studied the volatiles formation of thermal (heat sterilization and blanching) and HP–high-temperature treatments. They observed that the type of volatile varied as a function of treatment, and multivariate analysis could clearly classify the products based on the treatments. The volatiles from carrots are formed mainly by oxidative degradation and Maillard reaction. HP–high temperature enhanced oxidative degradation of terpenes, free fatty acids, phenylpropanoids, and carotenoids; and reduced Maillard reaction (i.e., lower furans) and Strecker degradation (i.e., lower aldehydes) products.

49.6.8 Low or Absence of Toxic Compounds

In many cases thermal treatment causes toxic compounds, such as acrylamide and furan. Furan (heterocyclic organic compound) is formed during thermal treatment of food products and it has been shown to be carcinogenic in animal laboratory studies [151]. Kultur et al. [80] studied the formation of furan during HP processing (pressure: 200, 300, and 400 MPa; temperature: 25°C, 35°C, and 45°C; treatment time: 5, 10, and 15 min) of vegetable-based infant food. At the end of processing the furan content in all HP-treated samples was found to be below the limit of detection. The allergenic effects of nuts could be reduced by HP treatment. HP treatment of walnuts (256 kPa, 138°C) were able to diminish the IgE cross-linking capacity more efficiently than heat treated walnuts [152]. HP treatment (200–600 MPa) showed any significant changes in the IgE binding in the case of beef extract [153], whereas bovine gamma globulin decreased [17]. In the case of apple allergen Mal d 3 neither thermal treatment alone (115°C) nor HP (700 MPa) alone caused any change, however, a combined treatment was most effective [154].

49.7 HIGH PRESSURE IN HURDLE TECHNOLOGY

The increasing demand for foods with reduced amounts of chemical additives and less physical damage is opening new opportunities for the hurdle-technology concept of food preservation [52, 56]. The commercial challenge of minimally processed foods provides a strong motivation to study food-preservation systems that combine traditional microbial stress factors or hurdles, while introducing "new" variables for microbial control, such as high pressure. High pressure presents unique advantages over conventional thermal treatments [76]. However, much of the reported data indicate that commercial pasteurization or sterilization of low-acid foods using high pressure is very difficult without using some additional factors to enhance the inactivation rate. Factors such as heat, antimicrobials, ultrasound, and ionizing radiation can be used in combination with high pressure. These approaches will not only help to accelerate the rate of inactivation, but can also be useful to reduce the pressure level and hence the cost of the process while eliminating the commercial problems associated with sublethal injury and survivor tails.

High pressure can be used to reduce the severity of the factors traditionally used to preserve foods. The use of high pressure in combination with mild heating has considerable potential [53]. Studies have shown that the antimicrobial effect of high pressure can be increased with heat, low pH, carbon dioxide, organic acids, and bacteriocins such as nisin [57, 72, 84, 155, 156]. Mackey et al. [62] observed that *L. monocytogenes* cells were sensitized to pressure by butylated hydroxyanisole, potassium sorbate, and acid conditions. Therefore, if the hurdle concept could be applied to the optimization of high hydrostatic pressure for low-acid foods, a combination of moderate treatments including pressure can lead to a food-preservation method effective against bacterial spores [72].

Enhanced pressure inactivation of microorganisms was observed when combining pressure treatments with additives such as acetic, benzoic, or sorbic acids; sulfites; some polyphenols; and chitosan [16, 76, 155, 156]. These combination treatments allow lower processing pressure, temperature, and/

or time of exposure. Roberts and Hoover [72] evaluated the effect of combinations of pressure at 400 MPa, heat, time of exposure, acidity, and nisin concentration against *B. coagulans* spores. Sublethally injured spores by the pressurization in combination with heat and acidity caused *B. coagulans* spores to become more sensitive to nisin. Acidic foods could be protected from spore outgrowth with the combined treatment. Hauben et al. [51] studied the lethal inactivation and sublethal injury of *E. coli* by high pressure and combinations of high-pressure treatments with lysozyme, nisin, and/or EDTA. High-pressure treatments from 180 to 320 MPa disrupted the outer membrane of bacterial cells, causing periplasmic leakage and sensitization to lysozyme, nisin, and EDTA, demonstrating that sublethal injury can be usefully applied in a hurdle technology approach as an effective food-preservation method.

Crawford et al., [83] evaluated the combination of high hydrostatic pressure, heat, and irradiation to eliminate *C. sporogenes* spores in chicken breast. These authors reported no significant differences in the number of surviving spores between samples that were first irradiated and then pressurized or vice versa. However, there was a significant difference between samples exposed to combined treatments and those that were only irradiated, with the combined processes being more effective. No survivors of the initial inoculated spores were observed with a 6 kGy irradiation dose followed by pressurization at 690 MPa and 80°C for 20 minutes. Crawford et al. [83] concluded that a combination of lower doses of irradiation and high pressure is more useful in eliminating *C. sporogenes* spores than the application of either process alone.

Earnshaw et al. [100] mentioned that there is no synergistic antimicrobial action between sorbic acid and pressure up to 400 MPa when applied to *Z. bailii* and attributed this lack of synergy to the modification of sorbic acid dissociation constant under pressurization. Tauscher [22] mentioned that the carboxylic acids commonly used as food preservatives show enhanced ionization when subjected to high pressure. However, Palou et al. [90] demonstrated that increased antimicrobial effects can be obtained when combining high pressure and potassium sorbate to inactivate *Z. bailii* in laboratory model systems with reduced a_w and pH. The initial inoculum (10^5 *Z. bailli* cfu/ml) was completely inactivated in systems with a_w 0.98 in the presence of potassium sorbate with pressures ≥345 MPa for more than 2 minutes; without potassium sorbate the pressure had to be applied at 517 MPa for 4 minutes. In laboratory systems with a_w 0.95 and without potassium sorbate, the pressure must be ≥517 MPa for 10 minutes. With potassium sorbate the treatment time could be reduced to 4 minutes [57].

Citric and sorbic acids were included in the laboratory model systems used by Palou et al. [90]. Thus, a temporary reduction in pH and an increase in the dissociated form of the acids could be present, which will depend on the pressure level. This effect could decrease the antimicrobial effectiveness of potassium sorbate during the time of exposure, since the major antimicrobial action is attributed to the undissociated form of the acid. However, the result of high-hydrostatic-pressure treatments would depend not only on the previously mentioned effects but on the consequences of high pressure in the biological systems involved. A synergistic antimicrobial action was observed between potassium sorbate and high pressure at both a_w [49]. For the same pressure level, the holding time required to inhibit *Z. bailii* is shorter in the presence of the preservative. High-pressure damage to *Z. bailii* renders cells more susceptible to other antimicrobial agents (low pH, potassium sorbate), probably due to the exposure of critical cell surface targets.

There is theoretical evidence about the pH shift during pressurization, but accurate models relating to microorganisms in food systems and direct measurement of pH are not available [100]. A detailed understanding of pH modification in foods might allow the design of food formulation that maximizes effective reversible antimicrobial pH shifts during pressurization [100]. The volume changes associated with ionization can be involved in the action of high hydrostatic pressure in biological systems. Earnshaw et al. [100] mentioned that water and acid molecules show increased ionization under high pressure. Thus, during the pressurization and holding time of a high-pressure treatment, an increase of proton concentration and a pH reduction are expected. High pressure decreases the pKa of certain acids that correspond to a decrease in the pH of solutions or buffers containing these acids [157]. Decreases in the pKa are more important for phosphoric acid than for citric acid. The pH of water or phosphate buffers decreases reversibly by 0.2–0.3 pH units per 100 MPa of applied pressure [158]. These effects can contribute, in a cooperative manner, to enhance the pressure effects on microorganisms, and the knowledge and understanding of their effects may aid to design effective pressure-combined processes. For most of the possible combined processes, the primary goal consists of identifying the factors or treatments that could sensitize microorganisms to pressure [18] or recognize the factors or treatments that could cause the microbial death in sublethal pressure-injured microbial cells. However, the protective effects that could exert food components made necessary the assessment of each combination process in each particular food product.

Cheftel [18] mentioned that potential applications of high pressure include decontamination of raw milk and some curds and cheeses made from raw milk; reduction of the intensity of thermal processing for prepared chilled meals containing thermosensitive food constituents; and the sanitation and increase of the refrigerated shelf life of spreads, emulsified sauces, essential oils, aromatic extracts, and herbs.

49.8 REGULATORY ASPECTS OF HIGH PRESSURE

No specific official requirements are needed in some countries that relate to the control of new food-processing technologies [8]. However, the regulations of food safety are relevant, and there are still several important questions about safety and nutritional value that need to be answered.

Earnshaw [8] mentioned that it is unlikely that pressure processing will replace canning or freezing, nevertheless it

could find applications for expensive foods with short shelf lives and high-value ingredients such as flavors, vitamins, and functional biopolymers that are heat-sensitive. In Europe, Japan, and the United States there is significant commercial interest in the development of high-pressure food processing, and millions of dollars have been invested in research and development.

High hydrostatic pressure is not a cheap technology, and a systematic approach must be taken to search for processing options to ensure that high-pressure treatments can be successfully and economically applied to a wide range of products [48]. The feasibility studies must include effective equipment design solutions and precisely defined minimum required pressures and time cycles. Continuous operation is also a major task.

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50 Applications of Magnetic Field in Food Preservation

Jasim Ahmed and Hosahalli S. Ramaswamy

CONTENTS

50.1 INTRODUCTION

Thermal energy induces various biochemical reactions, leading to quality deterioration in foods resulting in undesirable changes in nutritional and sensory characteristics [1, 2]. Therefore, emerging and novel technologies can overcome the limitations of thermal processing and draw significant attention from food processors as well as consumers. Increasing consumer demand for newer food products with health benefits, desirable sensory characteristics, and higher quality retention has also led to the development of mild preservation technologies or minimally processed foods.

In the past, several mild preservation techniques have been developed and tested. Gamma irradiation is one of those. Although the technique has been successful in decontaminating food, consumer acceptability is significantly low for this process. In recent years, the advents of some nonthermal processing technologies that pasteurize and/or sterilize foods have been emerging as alternatives to conventional thermal processes. Most of these novel process technologies focus on energy-saving and eco-friendly applications. These processes have a similarity as they are mild for the food, and kill only the targeted spoilage and pathogenic microorganisms. The food product in question retains most of its natural appearance. In addition, these technologies are of specific interest to the food industry, because they provide attractive alternatives to conventional methods of thermal processing, which often cause undesirable changes in foods. These alternatives could also provide high quality with improved processing economy without compromising safety, and offer

opportunities for creating new ingredients and products due to their specific actions on biological materials and food constituents [3].

Some of the potential mild preservation techniques are (i) high hydrostatic pressure, (ii) pulsed electric fields, (iii) oscillating magnetic fields, (iv) high-intensity light pulses, (v) electron-beam radiation, (vi) microwave and radiofrequency, (vii) ohmic and inductive heating, (viii) high-voltage arc discharge, (ix) ultrasound, (x) X-ray. Among these listed, some of these technologies are now commercially exploited, and few of them are still in the developmental stages. The application of magnetic fields to process food products is one of these novel non-thermal processing methods, and so far a limited number of studies have been carried out on the application and commercialization of this technology. The process could produce fresh-like attributes of foods by retaining thermo-labile nutrients with reduced energy requirements, and could be applied in flexible packages [4]. In the last few years, electromagnetic freezing has received tremendous attention because of its claim of good quality by producing the finest ice crystals. The application of magnetic fields in the food fermentation industry could be a major success by controlling cellular growth and inhibition.

50.2 BASICS OF MAGNETISM AND MAGNETIC FIELD

50.2.1 Magnetism

Magnetism is a phenomenon by which materials exert an attractive or repulsive force on other materials. The origin of magnetism lies in the orbital and spin motions of electrons, and how the electrons interact with each other. The understanding of materials' responses to magnetic fields is important to apply different types of magnetism. The materials are mostly classified as diamagnetic, paramagnetic, and ferromagnetic according to their responses to magnetic fields.

Diamagnetism is a fundamental property of all matter, though it is usually very weak. It results from the non-cooperative behavior of orbiting electrons during exposure to a magnetic field. Diamagnetic substances are made of atoms that do not have any net magnetic moments (all the orbital shells are filled and there are no odd electrons). Water is a good example of a diamagnetic material/fluid. However, when exposed to a field, a negative magnetization is produced; thus the susceptibility is negative. In paramagnetic materials, some of the atoms or ions have a net magnetic moment due to unpaired electrons in a partially filled orbital. Oxygen is the best example of unpaired electrons. However, the individual magnetic moments do not interact magnetically, and similar to diamagnetism, the magnetization becomes zero when the field is taken out. In the presence of a field, there is now a partial alignment of the atomic magnetic moments in the direction of the field in ferromagnetic materials, resulting in a net positive magnetization and positive susceptibility. Unlike paramagnetic materials, the atomic moments in ferromagnetic materials exhibit very strong interactions. These interactions are developed by electronic exchange forces resulting in a parallel or antiparallel alignment of atomic moments. Exchange forces are very large, equivalent to a field in the order of 1000 Tesla, or approximately 100 million times the strength of the earth's field. The exchange force is a quantum mechanical phenomenon due to the relative orientation of the spins of two electrons. Ferromagnetic materials exhibit parallel alignment of moments resulting in large net magnetization even in the absence of a magnetic field. The most common examples of ferromagnetic materials are iron, nickel, cobalt, and some steels.

50.2.2 Magnetic Fields

A magnet is surrounded by an invisible force field. A magnetic field is a region of magnetic force generating out from a permanent magnet. Magnetic fields are created by moving charged particles: in electromagnets, electrons flow through a coil of wire connected to a battery; in permanent magnets, spinning electrons within the atoms generate the field (Figure 50.1). The strength of the magnetic field is a direct function of current passing in the wire. An electromagnetic (EM) field contains both an electrical and a magnetic field. In the case of a fluctuating magnetic or EM field, the field is characterized by its rate, or frequency of fluctuation (e.g., one fluctuation per second is equal to 1 Hz, the unit of frequency). Lines of magnetic force can be seen around a magnet by sprinkling iron filings onto a sheet above it and tapping the sheet. The strength of the magnetic force is strongest nearest to the poles and gets weaker while the poles move away from each other. A magnetic field can also be created by the spin magnetic dipole moment, and by the orbital magnetic dipole moment of an electron within an atom. A magnetic field is a vector field: it associates with every point in space and a vector may vary in time.

50.2.3 Magnetic Field Lines

Magnetic field lines can be used to visualize the magnetic field clearly. The magnetic fields generated by a single magnet are illustrated in Figure 50.2. The distance between those lines indicates the strength of the field when drawn properly. The closer the distance, the more strong the field. The number of lines per square centimeter measures the strength of the magnetic field. Technically, 1 Gauss is equivalent to one

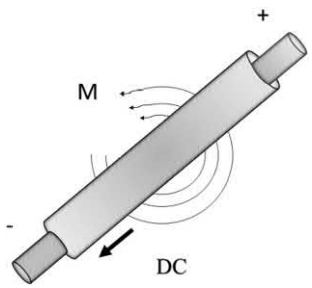

FIGURE 50.1 Current flowing through a wire produces a magnetic field (M) around the wire.

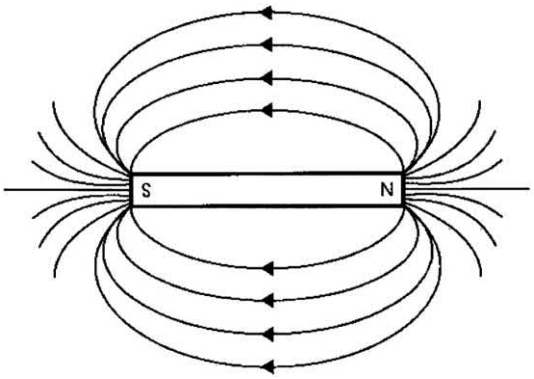

FIGURE 50.2 Single magnet exhibiting magnetic field.

magnetic field line within 1 square centimeter, and the direction of the tangent to the field line is the direction of the magnetic field at that point.

50.2.4 Symbols and Terminology

The fundamental vector quantities describing a magnetic field are the magnetic field strength H and the magnetic flux density B (also called the magnetic induction). The relationship between magnetic flux density, B, and the magnetic field strength, H, is given by $B = \mu H$ which is used to describe the magnetic field generated by currents that flow in conductors. The value of μ (the magnetic permeability) is determined by the properties of the medium. For most biological materials, the permeability μ is equal to μ_0, the value of permeability of free space (air) (1.257×10^{-6} H/m). Thus, for biological materials, the values of B and H are related by the constant μ_0.

Magnetic fields, like electric fields, are produced by electric charges, but only when these charges are in motion. Magnetic fields exert forces on other charges but, again, only on charges that are in motion. The magnitude of the force F acting on an electric charge q moving with a velocity v in the direction perpendicular to a magnetic field of flux density **B** is given by:

$$\mathbf{F} = q\mathbf{v} \times \mathbf{B} \tag{50.1}$$

Equation 50.1 is known as the Lorentz force law. The direction of **F** is perpendicular to both those of **v** and **B**. If, instead, the direction of **v** was parallel to **B**, then **F** would be zero. This indicates an important characteristic of a magnetic field: it does no physical work, because the force, called the Lorentz force, generated by its interaction with a moving charge is always perpendicular to the direction of motion.

Magnetic fields, like electric fields, are produced by electric charges, but only when these charges are in motion. Magnetic fields exert forces on other charges but, again, only on charges that are in motion. The magnitude of the force F is acting on an electric charge q moving with a velocity v in the direction perpendicular to a magnetic field of flux density.

The basic unit of the magnetic flux density can be obtained from Equation50.1 to be Newton second per coulomb meter (N s/C m). In SI units, B and H are measured in Teslas (T) and amperes per meter (A/m), respectively, or, in cgs units, in gauss (G) and oersteds (Oe), respectively. The conversion between the gauss (G), the cgs unit of flux density, and the tesla is 1 T = 10^4 G. A simpler form of the force equation in a wire current loop is given by:

$$F = BLi = (\text{Tesla}) \times (m) \times (\text{amp})$$

A more complex explanation is that if the moving charge is part of a current in a wire, then an equivalent form of the law is given below:

$$\frac{d\mathbf{F}}{dl} = \mathbf{i} \times \mathbf{B} \tag{50.2}$$

In words, Equation 50.2 infers that the force per unit length of wire is the cross product of the current vector and the magnetic field. In the equation above, the current vector, **i**, is a vector with magnitude equal to the usual scalar current, *i*, and direction pointing along the wire that the current is flowing.

50.2.5 Types of Magnetic Field

Magnetic fields may be homogeneous or heterogeneous and can be in static and pulsed mode. In a homogeneous magnetic field, the field intensity is uniform in the area enclosed by the magnetic field coil, while in a heterogeneous field, the field intensity is non-uniform and field intensity decreases as distance from the center of the coil increases. Static magnetic fields (SMF) show a constant strength over time and are generated by permanent magnets or direct current electromagnets. An oscillating magnetic field (OMF) is applied in the form of constant amplitude or decaying amplitude sinusoidal waves and exhibits an intensity gradient over time depending on the nature of the magnet. OMFs are generated by alternate current electromagnets within pulsed fields, and the field intensity alters periodically depending on the frequency and type of wave generating from the electric current in the magnet [5]. The magnetic fields have been classified again as high- or low-intensity fields as per their relative intensity. Low-intensity magnetic fields show strength in the order of one tenth of a gauss, while high-intensity fields show strength in the order of thousands of gauss or greater [6].

50.3 MAGNETIC FIELD GENERATION

50.3.1 Field Generation by Currents in Wires

The simplest way to create a magnetic field is by supplying current along a long straight wire. The magnetic field from such a current-carrying wire actually wraps around the wire in circular loops, decreasing in magnitude with increasing distance from the wire. The magnitude of the field at a distance *r* from a wire carrying a current *I* is given by:

$$B = \mu_o I/(2\pi r) \tag{50.3}$$

where μ_o is a constant, the permeability of free space and $\mu_0 = 4\pi \times 10^{-7}$ T m/A, T is tesla. The magnetic action to inactivate microorganisms has some required specific field strengths, which vary between 5 and 50 T. The generation of such field strength is carried out by (i) superconducting coils, (ii) coils which generate DC fields, and (iii) coils that can be energized by the discharge of the energy stored in a capacitor [7]. Magnetic fields generated by iron core in coil cannot produce the required intensities for microbial inactivation and therefore are not suitable for this purpose. The air-core solenoids can produce higher intensities, and the intensity is directly related to the flow of current, which is found to be a limiting factor due to huge power consumption and heat generation [5].

50.3.2 Static Magnetic Fields Technique

Superconducting magnets are a good choice for high-intensity magnetic field generation since they avoid heating effects. Magnetic field generation with coils of super-conducting metal has been used by various researchers. Metals behave as super-conductors (electricity passes through the wire with no resistance) in a liquid helium environment (–268.9°C). This generates a magnetic field of about 2 T, which is about 40,000 times stronger than the earth's magnetic field (0.0005 T). The liquid helium is insulated by a dewar (insulated storage vessels) of liquid nitrogen, which helps to reduce the loss of helium from the magnets. The magnets need to be filled with liquid nitrogen regularly (weekly), and liquid helium needs to be filled about once a month, which makes this type of magnet expensive to run. However, it has also some limitation in field intensity, with maximum limits of about 20 T. A hybrid magnet contains a superconducting magnetic coil and water-cooled magnetic coil that can produce 15–30 T. Magnetic fields above 30 T have been generated in a pulsed form by supplying a current of 40 kA for short time period [8].

50.3.3 Oscillation Magnetic Fields Technique

Considerable effort has been made to generate high-intensity magnetic fields to extend the available field range. MAGNEFORM® magnetic forming systems (Magneform, San Diego, CA) have achieved wide acceptance as a proven production method on today's high-volume assembly lines. Magneform machines have been routinely used to form, join, or assemble parts of metals and have been tested for food processing applications. Hofmann [9] was probably the first one to test the Magneform 7000 for food applications, and such equipment has been used more recently by Harte et al. [10]. The working principles and coil circuits have been well-described by Pothakamury et al. [5]. The instrument can store the energy in a capacitor bank. The basic principle of magnetic pulse is the same, as it activates a simple electric motor. An electric current generates a pulsed oscillating magnetic field (OMF) between the plates of the capacitors where the food sample is held in plastic bags. The frequency of the magnetic field is dictated by the capacitor's capacitance and the resistance, and induction of the coil. An OMF of 2–50 T is achieved while a coil has been connected to a capacitor of 8–16 kJ [9].

50.3.4 Ultra-High Magnetic Fields

The generation of higher fields (in the megagauss ranges) is still difficult because of the coil destruction by the higher electromagnetic forces. Miura et al. [11] have developed ultra-high magnetic fields in the megagauss range using the electromagnetic flux compression technique and direct fast current discharge into a single turn coil. The authors have claimed the novel processes can generate 500–1000 T field strength.

Scientists from National High Magnetic Field Laboratory, University of Florida, have recently developed [12] one superconducting magnet, which stands 16 feet tall and weighs more than 15 tons. Now, with its commissioning, scientists from around the world will be able to expand the horizons of scientific investigation using nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) technologies. The bore is the space within the magnet that holds the sample being tested. The bore size of this magnet (105 mm) makes it particularly useful for scientific research. The 900 MHz magnet delivers 21 T magnetic fields that vary less than 0.0000002 T over a volume which is roughly equal to the size of a small orange.

50.4 APPLICATION OF MAGNETIC FIELDS IN FOOD PRESERVATION

50.4.1 Pasteurization

The success of food preservation basically depends on the inactivation of enzymes and microorganisms which would produce safe food for human consumption. The microbial growth can either be stimulated or ceased during the exposure to magnetic fields. The retardation of growth and reproduction of microorganisms might be associated with changes in DNA synthesis; a change in the orientation of biomolecules and biomembranes to a direction parallel or perpendicular to the applied magnetic field; or a change in the ionic drift across the plasma membrane [5].

The first approach to food preservation by OMF was initiated by Hofmann [9] with a US patent. The basic requirement for the application of OMF technology for food preservation requires a high electrical resistivity, greater than 10 to 25 ohm/cm. Many foods have electric resistivity in these ranges. One of the most common examples of a food that can be processed by the OMF technique is orange juice. The electrical resistivity of orange juice is about 30 ohm-cm. The field intensity is a function of electrical resistivity and sample thickness. However, no correlation has been observed between magnetic field intensities and food constituents.

The inactivation of microorganisms in food has been reported to occur during exposure to OMFs with intensity higher than 2 T [9]. A single pulse intensity of 5–50 T and frequency of 5–500 kHz reduced the initial number of

microorganisms by two log cycles. The technology could be applied for food pasteurization purposes by placing the sample in a magnetic field and magnetizing. The author reported the application of OMF for selected food products to control specific spoilage microorganisms as (i) milk with *Streptococcus thermophilus*, (ii) yogurt with *Saccharomyces cerevisiae*, (iii) orange juice with *S. cerevisiae*, and (iv) Brown 'N Serve roll dough with bacterial spores. These studies also indicated that the temperature increase during the magnetic field is almost negligible (maximum 2°C), and the reduction of microorganisms ranges between 1.4 and 3.6 log cycles.

50.4.2 Microbial Systems

50.4.2.1 Inactivation of Microorganisms

Almost all living organisms are exposed to magnetic fields from different sources. The geomagnetic field on the surface of the earth is approximately 0.50–0.75 gauss in strength. When a magnetic field is applied to microorganisms in a liquid culture, it is distorted or weakened by that culture. A strong field may significantly affect microorganisms, or even kill them. The effects of magnetic fields on microbial growth have been classified by Yoshimura [13] as inhibitory, stimulatory, and none observable.

Several studies have been carried out on the effects of exposure to magnetic fields, and many of these findings are inconsistent (Table 50.1). Hoffman [9] first advocated that exposure to magnetic fields causes inhibition by reducing the growth and reproduction of microorganisms. Exposing food infected with microorganisms to an OMF at frequencies of 5–50 kHz and with an electro-magnetic strength of 5–50 T reduced the microorganisms' population by 2 to 3 log cycles. The formation of metastable pores by the presence of natural magnetite or contaminant magnetic particles on cell membranes has been considered an effect of magnetic fields [14]. The growth rate of the Burgundy wine yeast has been reported to decrease at an extremely low magnetic flux density of 0.04 T [15]. On the contrary, the accelerated growth rate of Trichomonas vaginalis was reported when exposed to 46–120 T [16]. The growth rate of Bacillus subtilis was also found to increase when exposed to 0.15 T and decrease when exposed to more than 30 T [17]. Similar results were reported for Chlorella; an exposure of less than 400 gauss increased the growth, while exposure to 580 gauss decreased the growth rate [18]. Several studies pointed to the magnetic fields as a factor influencing the growth and survival of living organisms, which vary at different magnetic flux densities [19, 20–22].

The strength of the magnetic field has significant effects on microbial growth and survival. The cellular growth in the stationary phase of *Escherichia coli* under a heterogeneous magnetic field of 5.2–6.1 T was found to exhibit a significant increase in the number of cells by about 10^5 as compared to the geomagnetic field [23]. The authors claimed that such a marked death suppression effect by the high magnetic field had never been reported earlier. The amount of sigma S factor encoded by the *rpoS* gene under a high magnetic field was 20% higher in comparison to control geomagnetic field which might be part of the reason why a large number of cells in the stationary phase were maintained under the heterogeneous magnetic field. Fojt et al. [24] studied biological effects of low-frequency electromagnetic fields using three different bacterial strains, namely *E. coli*, *Leclercia adecarboxylata*, and *Staphylococcus aureus*. The strength and frequency of

TABLE 50.1
Effect of Magnetic Fields on Microorganisms

Type of Microorganisms	Nature of Magnetic Fields	Field Strengths (T)	Major Finding	References
Burgundy wine yeast cells	Static	0.04	Growth retardation during exposure at 5–150 min; no retardation at 10, 15, and 17 min	Kimball [14]
Trichomonas vaginalis	Static	4.60–12	Growth was accelerated	Genkov et al. [15]
E. coli	Oscillation	0.05	Inactivation of cells when concentration was 100 cells/mL	Moore [16]
Streptococcus thermophilus in milk	Oscillation	12.0	Cell population reduced from 25,000 to 970 cells/ml	Moore [16]
Saccharomyces in yogurt	Oscillation	40.0	Cell population reduced from 3,500 to 25 cells/ml	Hofmann [9]
In orange juice	Oscillation	40	Cell population reduced from 25,000 to 6 cells/ml	Hofmann [9]
Mold spores	Oscillation	7.5	Population reduced from 3,000 to 1 spores/ml	Hofmann [9]
E. coli	Static	5.2–6.1	Magnetic field increased viable cells by 150 times after incubation for 48 h	Horiuchi et al. [22]
E. coli, *Leclercia adecarboxylata*, and *Staphylococcus aureus*	Oscillation	0.01	Magnetic field caused the decrease of CFU in all exposed samples. The maximum decrease of CFU was observed for *E. coli*; *S. aureus* strain was found most resistant to the magnetic field	Fojt et al. [23]
Saccharomyces cerevisiae	Static	5–14	Rate of proliferation under the magnetic fields decreased after 16 h of incubation compared to control sample	Iwasaka et al. [34]

the field were 10 mT and 50 Hz, respectively, whereas the residence time was less than 30 min. The low magnetic field caused a decrease in CFU in all exposed samples. Viability decreased with longer exposure time and/or higher induction fields for all strains, but the effect was strain-dependent. The highest decrease of the viability and the largest magnetic field effect was observed with *E. coli* while the lowest magnetic field effect was found for *S. aureus*. From the measurement of the growth dynamics, it was concluded that the decrease of the CFU started immediately after the magnetic field was applied. However, Harte et al. [10] found no significant reductions in *E. coli* under magnetic fields lower than or equal to 18 Tesla generated by both OMF and SMF using a 7000 series Magneform machine (Maxwell Laboratories, CA) and a superconducting magnet (National High Magnetic Field Laboratory, NM), respectively.

The finding of enhanced viability of cells under a high magnetic field implies that the productivity of antibiotics or enzymes by microorganisms is enhanced under a high magnetic field. It is already reported that the high magnetic field has no mutagenic or adverse effect on biological cells [25].

50.4.2.2 Mechanisms of Microbial Inactivation

Several hypotheses have been postulated for the mechanism of microbial inactivation by magnetic action. The inactivation of microorganisms may be based on the theory that the OMF may couple energy into the magnetically active parts of large molecules like deoxyribose nucleic acid (DNA) [9]. Within the 5–50 T range, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} to 10^{-3} eV. Pothakamury et al. [5] described two theories on the microbial inactivation process using either SMF or OMF:

(i) A weak OMF could weaken bonds between ions and proteins used in metabolism and membrane integrity. In SMF such as the earth, the biological effects of OMF are more pronounced around particular frequencies, the cyclotron resonance frequency of ions [26].
(ii) The effect of magnetic fields on calcium ions bound in calcium-binding proteins like calmodulin. The Ca^{++} ions vibrate regularly to an equilibrium position in the binding site of calmodulin. A steady magnetic field to calmodulin results in the plane of vibration to rotating, or proceeding in the direction of the magnetic field at a frequency that is exactly similar to the cyclotron frequency of the bound calcium. Adding a "wobbling" magnetic field at the cyclotron frequency disturbs the precision to such an extent that it loosens the bond between the calcium ion and the calmodulin [5].

The results presented in Table 50.1 show that, although not well understood, the effect of magnetic fields on the microbial population of foods may depend on the magnetic field intensity, number of pulses, frequency, and property of the food (that is, resistivity, electrical conductivity, and thickness of the foodstuff). Unfortunately, studies on the effects of magnetic fields on microorganisms have produced conflicting results. Until more consistent and convincing results are produced, the prospects for this technology are questionable.

50.4.2.3 Propagation of Yeast

Studies concerning the use of *S. cerevisiae* as a cell model have reported that proliferation increases more than 25% at 50 Hz, 0.5 mT alternating magnetic fields (MF) during 10 h exposure [27]. Nevertheless, the effect observed by these authors when they used 0.2 mT, 50 Hz, in the exposition is the inhibitory action (16%) of cell proliferation. The cell growth dependence with field frequency found by them exhibits positive responses by a more than 20% increase in the cell number after 10 h of exposure to 15 and 50 Hz. However, conflicting results have been reported by Ruiz-Gomez et al. [28] while studying the growth induced by static and sinusoidal 50 Hz magnetic fields (MF) on the haploid yeast strain *S. cerevisiae*. Magnetic fields were produced by a pair of Helmholtz coils (40 cm in diameter) with 154 turns of copper wire in each and separated by 20 cm. The experiments were carried out at 0.35 and 2.45 mT, and yeasts were exposed to MF for time periods of 24 and 72 h in the homogeneous field area. Growth was recorded by measuring the optical density at 600 nm. They reported that static and sinusoidal 50 Hz MF (0.35 and 2.45 mT) did not induce changes in the growth of *S. cerevisiae*.

50.4.2.4 Yeast under High-Gradient Magnetic Field

Superconducting technology has provided new methods for studying the application of strong magnetic fields in biological systems and dia- or paramagnetic materials. Room temperature bores of superconducting magnets have given opportunities to explore the importance of the diamagnetism of biological materials [29, 30] as well as that of strong (ferro-) magnetism in solid-state materials. A new approach using superconducting technology involved gradient magnetic fields in the horizontal direction which led to several interesting phenomena. It was reported that the surface of water bent downward under the magnetic field of 10 T or so at the center [31]. The parting of water has been termed as the "Moses Effect," a phenomenon in which the surface of water has been separated under high magnetic fields [32]. The horizontal gradient magnetic force and the vertical gravitational force generate a sloping gravity-like field for living organisms [33], in which the sloping field is considered to be the actual direction of gravity. In the case of the parting of water, the water was pushed to lower magnetic fields from higher fields, and localized in the middle part of the space forming a "water-wall." However, the applied field should be high and steep enough in order to clearly observe these effects.

The effects of gradient magnetic fields on the behavior of yeast (*S. cerevisiae*) proliferation and mass distribution have been reported also by Iwasaka et al. [34] using strong static magnetic fields (flux density 14 T). When yeast was exposed to 9–14 T magnetic fields with a maximum flux density gradient of $dB/dx = 94\,\mathrm{T}/m$, where x is the space coordinate, the

rate of yeast proliferation under the magnetic fields decreased after 16 h of incubation as compared to that of the control. The results indicated that the gas pressure inside a flask with 6 T, 60 T/m gradually increased in comparison to the pressure inside a control tube. Due to the diamagnetism of medium water and yeast, the liquid surface clearly inclined under gradient magnetic fields, and the hydrostatic force in suspension was strengthened by the diamagnetic forces. In addition, magnetophoresis of the yeast cells in the medium solution exhibited localization of the yeast sedimentation pattern. The mechanisms for deceleration of yeast proliferation by magnetic fields have been proposed and well-described by Iwasaka et al. [34].

50.4.3 Sterilization by Magnetic Fields

Complete sterilization is possible by exposure of magnetic fields to more than one oscillation sequence [35]. Microorganisms tested are viruses, bacteria, mold, protozoa, and algae. In this technique [35], a low electrical conductivity material is passed through a high-intensity, moderate-frequency oscillating magnetic field for a very short residence time, which is adequate for inactivating a major portion of the microorganisms within or upon the material to achieve substantial sterilization without detectably altering the material itself. The decaying magnetic oscillations can be produced by discharging voltage from a capacitor into a coil so that the capacitor and coil are in an electrical loop so that no other components will deter from the natural back-and-forth oscillations. An output of approximately ten magnetic pulses (five each from north and south poles) is significant in strength because each pulse becomes weaker than the one before due to energy dissipation in the wire, coil, and capacitor. The best range of initial magnetic field strength is 5 to 50 Tesla, but anywhere between 2 and 100 will work [35]. Generally, higher electrical conductivity of the infected matter requires less magnetism strength to inactivate the microbes. Foods with minimal electrical conductivity need to be exposed to 10–100 oscillation sequences for complete sterilization. It is believed that some microbes are killed while the majority are just devitalized so that they can't reproduce [35].

50.4.4 Effects of Magnetic Fields on Food Freezing

Magnetic freezing has received serious attention both from the food industry and academics, recently, as a commercial reality. in the last two decades, plenty of patents have been filed, and many companies marketed electromagnetic freezers that apply different types of magnetic fields to improve the quality of frozen food [36]. A few commercial companies are marketing electromagnetic freezers including ABI Co., Ltd. (Chiba, Japan) which sells "CAS (Cells Alive System) freezers" combining static and oscillating magnetic fields; and Ryoho Freeze Systems Co., Ltd. (Nara, Japan) which sells "Proton freezers" that use static magnetic fields and electromagnetic waves.

In electromagnetic freezers, permanent magnets and induction coils are used to produce a weak oscillating magnetic field within the freezing chamber [37]. CAS is not a refrigeration process itself, but is employed to assist in improving existing freezing processes (both in terms of process speeds and product quality). A typical CAS freezing system is illustrated in Figure 50.3 as described by Otero et al. [36]. The freezer consisted of a cooling unit, two fans, a control panel, and a freezing cabinet (Figure 50.3). The cabinet consisted of 10 equidistant rails with a rack that can accommodate up to 10 trays for food freezing and the magnetic field generators. The static and oscillating magnetic field generators are employed to assist the freezing process. The static magnetic field is produced by a number of permanent magnets embedded in the front door and in the ceiling, the floor, and the left and rear walls of the freezing cabinet; the OMF is generated by four rectangular magnetic coils located inside the cabinet. The patent literature pertaining to CAS indicated that the oscillating magnetic field acts on polarized water molecules to delay the formation of ice crystals [38, 39]. Furthermore, it is claimed that oscillating magnetic field–induced freezing is able to generate fine ice crystals throughout the frozen product, prevent cell destruction, improve heat transfer, and retain

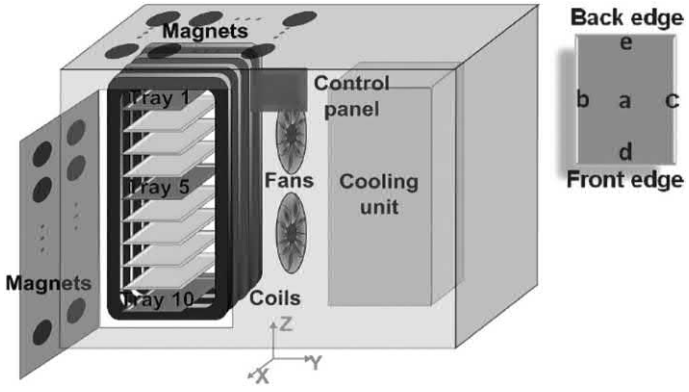

FIGURE 50.3 Typical schematic diagram of the CAS freezer: the main component (left) and the points at which magnetic field measurements were done in selected freezing trays 1, 5, and 10.

the quality of fresh food after thawing [39, 40]. However, it is still in question whether the extremely low strength of the OMFs commonly applied in commercial freezers (<2 mT) has this capability on a substance with a low magnetic susceptibility such as water. Additionally, the mechanisms cited in the patents to explain the effects of OMFs on water molecules are ambiguous, not scientifically proved, and, according to Kobayashi and Kirschvink [41], "do not agree with basic biophysics."

Otero et al. [36] assessed the effectiveness of OMFs in preserving crab sticks in a commercial electromagnetic freezer, both with (<2 mT, 6–59 Hz) and without OMF application. It was found that the OMFs did not have any effect on the drip loss, water-holding capacity, toughness, and whiteness of the crab sticks frozen in the electromagnetic device, and stored for a year. A similar study was carried out by James et al. [42] on individual cloves within whole garlic bulbs using CAS, and its effect on the degree of super-cooling and characteristic freezing curve as compared to freezing under the same conditions without OMF. Overall, it was observed that freezing under the OMF conditions had little significant additional effect on the freezing characteristics, or degree of super-cooling of garlic bulbs, in comparison with freezing under the same environment without OMF. More experiments at higher magnetic field strength and wider frequency ranges should be carried out to establish the potential benefits of OMFs on food freezing.

Magnetic resonant freezing (MRF) is considered as another potential freezing technique that could possibly increase the quality of food material due to uniform flash freezing of the entire food volume by producing fine ice structure in food. The MRF process consists of two stages: (i) food undergoes continuous magnetic wave vibrations, which impede the crystallization, and (ii) instantaneous removal of the magnetic field, which leads to freezing [43, 44]. Changes in the ultrastructure and quality of beef muscle during frozen storage by MRF and air blast freezing and the changes in the quality characteristics after thawing were compared by Choi et al. [45].

It was observed that the size of the ice crystal was minute and evenly formed in the initial freezing period; however, the size was increased as the storage period elapsed. The beef stored by the electro-magnetic resonance freezing exhibited a lower rate of growth in the ice crystal size as compared to the air blast freezing during the frozen storage. The thawing loss of beef stored by the MRF was significantly lower than the air blast freezing, and it showed that the thawing loss of the round was higher than the loin. The sensory evaluation indicated that the beef stored by the MRF did not show the difference until 4 months, and it showed higher acceptability in comparison with the beef stored by the air blast freezing. Therefore, the freezing method has an effect on the change in the ultrastructure and quality characteristics of the beef.

50.4.5 Biological System

Henry Lai and colleagues [46] at the University of Washington have discovered a method of treating malaria with magnetic fields that could prove revolutionary in controlling the disease which the World Health Organization calls one of the world's most complex and serious human health concerns. Researchers claim the malaria parasite *Plasmodium* appears to lose vigor and can die during exposure to oscillating magnetic fields, which may cause tiny iron-containing particles inside the parasite to move in ways that damage the organism. The oscillating magnetic field may affect the parasites in two ways. In organisms still in the process of binding free heme molecules into stacks, the alternating field likely "shakes" the stacked heme molecules, preventing further stacking. That would allow harmful heme free reign within the parasite. If the parasite is further along in its life cycle and has already bound the heme into stacks, the oscillating field could cause the stacks to spin, causing damage and death to the parasite.

50.4.6 Isolation and Separation of Protein by Magnetic Technique

The isolation and separation of specific molecules is common practice in almost all areas of biosciences and biotechnology. Various techniques have been used to achieve this goal. Recently, increased attention has been paid to the development and application of magnetic separation techniques, which employ small magnetic particles. Safarik and Safarikova [47] recently reviewed magnetic techniques for the isolation and purification of proteins and peptides.

The basic principle of batch magnetic separation is simple. Magnetic carriers bearing an immobilized affinity or hydrophobic ligand or ion-exchange groups, or magnetic biopolymer particles having an affinity with the isolated structure, are mixed with a sample containing the target. Magnetic separation techniques have several advantages over standard separation techniques. This process requires only a few handling steps. The separation can be carried out directly in crude samples containing suspended solid material. Due to the magnetic properties of magnetic adsorbents (and the diamagnetism of most of the contaminating molecules and particles), they can be relatively easily and selectively removed from the sample. In fact, magnetic separation is the only feasible method for recovery of small magnetic particles (diameter of 0.1–1 μm) in the presence of biological debris and other fouling material of similar size. In addition, the power and efficiency of magnetic separation procedures are useful in large-scale operations. Several automated systems for the separation of proteins or nucleic acids have become available recently.

Magnetic separation is usually a very mild operation on the target proteins or peptides. Even large protein complexes may remain intact when using the very gentle magnetic separation procedure [48]. The separation process has been significantly influenced by both the reduced shearing forces and the higher protein concentration throughout the isolation process. Appropriate magnetic particles can be used for their concentration instead of ultrafiltration, precipitation, etc. [49].

50.5 APPLICATIONS OF OTHER MAGNETIC PROPERTIES IN FOOD QUALITY SYSTEMS

50.5.1 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a unique technique for real-time and in-situ measurements due to its non-invasive and non-perturbing nature, which makes it suitable for quality control of food products. This is of particular advantage for in vivo measurements, and also for the assessment of phenomena that occur during the processing and storage of food products. MRI is based on the principles of nuclear magnetic resonance (NMR), a spectroscopic technique used by scientists to obtain microscopic chemical and physical information about molecules. However, the tremendous potential of magnetic resonance systems in other fields has not been fully explored yet. The technique was called magnetic resonance imaging rather than nuclear magnetic resonance imaging (NMRI) in order to avoid negative connotations associated with the word nuclear in the late 1970s [50].

50.5.2 Application of MRI in Quality Control of Food Products

MRI is an attractive tool for temperature mapping (distributions) induced in water-based foods by microwave and conductive heating. Additionally, it can measure quantitatively in three dimensions all aspects of the mass transport of water and/or fat in foods to give direct information about the effects of process engineering, including flow, mixing, and heating.

MRI has been proven efficient as an in-line sensor for detecting defects and measuring the quality of fruits and vegetables. MRI can predict the structure and dynamics of foods and other heterogeneous materials during and after processing. MRI has been used efficiently as a viscometer; MRI measurements of the fluid velocity profile in tube flow are coupled with a pressure drop measurement to yield shear viscosity. Each measurement yields a range of shear viscosity data for a single flow rate, as compared to most conventional viscometers that produce only one data point under the same circumstances. This technique has the potential to significantly enhance process control of industrial processes.

50.5.3 Application of NMR in Quality Control of Food Products

NMR is used extensively as an analytical tool in the characterization and quality control of food products. NMR profiles can detect overall food compositions by assessing the effects of agricultural production and harvesting, storage, and processing [51]. The water mobility in food products can be traced through relaxation spectra involving the NMR-active nuclei in water (^{1}H, ^{2}H, and ^{17}O). The amorphousness and crystallinity of starch can be detected by the solid-state NMR techniques. Mostly, dissolved or extracted samples are measured by liquid-state NMR, but in several cases the use of semi-solid-state ^{1}H HRMAS has also been applied to intact food materials. In several cases, site-specific natural isotope fractionation (SNIF)-NMR has become the method of choice for the detection of adulteration of food products with synthetic ingredients, such as flavors, sugars, and alcohols. The most significant application of NMR is the determination of authenticity of foods. This is a particular issue where biological or geographical origin, often in combination with artisanal manufacturing, has added significant value to food products [51].

50.6 CONCLUSION AND FUTURE RESEARCH NEEDS

The use of magnetic fields as an alternative food processing technology has been unable to gain full commercial acceptance, possibly due to inconsistent results on microbial growth and death kinetics. In addition, there is a significant lack of information on the field and the development of machinery. First, food scientists need to confirm that magnetic fields could effectively deactivate microorganisms. The application of magnetic fields could be effective and beneficial for biomass production at controlled growth rate using superconducting technology. This would help the growth of the fermentation and pharmaceutical industries. The structural modifications of proteins and fats could be achieved by exposing those constituents to magnetic fields. There is a significant lack of research in the food processing areas, and it is hoped that future development will provide answers to nagging questions. However, significant progress has been made in using NMR for quality assessment of foods, such as molecular mobility and structural changes, as well as identifying the effects of processing causing the changes in food materials.

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Part VI

Enhancing Food Preservation by Indirect Approach

51 A Glance at Nutrition

Mostafa Waly and Vickie A. Vaclavik

CONTENTS

51.1 INTRODUCTION TO PRESERVATION AND NUTRITION

This chapter focuses on the various preservation techniques and their effects on nutrition. Effective preservation of fresh food helps to ensure that foods contain desirable, nutritious components, and that these are safe and wholesome for consumption later. Precisely, the nutritive value of foods must be maintained during the process of preservation. Overall, in order to receive the health benefits of foods, nutrients must be present in the foods. Undoubtedly, each of the processing variables offers its own set of challenges. Cold preservation techniques result in less nutrient loss than heating as a means of preservation.

The two terms of preservation and processing are unique, and they could be interrelated. Briefly stated, food processing certainly includes preservation, and processing may include the use of specific food additives, some of which are known as "preservatives." Processing also includes food packaging treatments, which of course may assist in preserving food. Food preservation and processing could have beneficial or detrimental effects to the nutrients. For example, vitamin C is significantly lost by heating while the bioavailability of lycopene in tomato is higher when heated or processed as compared to fresh tomato.

51.2 NUTRITION

The inherent nutrient content of raw and fresh produce items declines with the passage of time. This decline could be due to improper handling, such as variations in post-harvest conditions, and preservation methods. Cooking or slow freezing of fresh food may typically result in nutrient loss but increases palatability and nutrient availability. "Nutrients" are chemical substances obtained from food, used for growth, maintenance, and repair of our body. The human body needs nutrients, and malnutrition may result from an under- or over-consumption of energy and nutrients. Cultural factors may influence the consumption of specific types of *foods* although populations of all cultures need the same *nutrients.*

The nutrients are grouped into six classes as macronutrients (i.e., water, 0 kcal/g; carbohydrate, 4 kcal/g; protein, 4 kcal/g; fat, 9 kcal/g), and micronutrients (i.e., vitamins, and minerals, with zero calories). The breaking of the structure of a macronutrient molecule produces energy, which may escape as heat, or build new compounds, as well as transport within the body.

The "essential" nutrients are those nutrients that cannot be made by the body at all or the amount produced is not enough. These essential nutrients are normally obtained from food and should not be dependent upon pills or other supplements. Personal preference is the most important reason why people choose their foods when different options are available. The Institute of Food Technologists reports that people choose foods based on appearance, texture, and flavor. Even children honestly give their opinions regarding these three attributes, as they remark on the foods before consumption. The nonnutrients are not included in the six classes, for example, the

phytochemicals. The six nutrients are discussed in the following sections of this chapter.

Foods may be nutritious with high levels of nutrients per calorie, or foods, many referred to as "junk foods" (a name often used since the 1950s), may have with low levels (or no amount) of nutrients per calorie. Among the six nutrients three offer calories, and three do not contain calories. Foods may be enriched or fortified with nutrients, as defined: enriched food contains added nutrients that are lost during processing, and fortified food contains added nutrients that are not present originally, for example, fortified breakfast cereals, and orange juice fortified with calcium.

51.2.1 Carbohydrate

Carbohydrates are the most important single source of food energy and their intake varies among countries depending on the culture and food recipes. Carbohydrate-containing foods provide up to 60% of total food energy, and these foods are root crops, pulses, vegetables, fruits, and milk products. Carbohydrates have the formula $C_n(H_2O)_n$, with a molar proportion of C:H:O of 1:2:1. Based on the number of monomeric units, carbohydrates are classified into sugars (1–2 units: monosaccharides, disaccharides, polyols), oligosaccharides (3–9 units: maltodextrins, raffinose, stachyose, fructooligosaccharidises), and polysaccharides (>9 units: amylose, amylopectin, cellulose, hemicellulose, and pectins) [1].

The major dietary carbohydrates are starch, sucrose, and lactose. Small amounts of free glucose and fructose are also present in the diet, in addition to glycogen and indigestible polysaccharides such as cellulose. Salivary α-amylase cleaves starch and glycogen by breaking at random the α-1,4 linkages between glucose residues within the chain leading to α-dextrins. α-amylase in saliva and the pancreas cannot hydrolyze: α-1,6 linkages, terminal α-1,4 linkages, and the 1,4 linkages next to branching points. Carbohydrates (a hydrated carbon—CH_2O) is one of the six nutrients used by the human body. It consists of numerous glucose units held together by glycoside bond linkages. Carbohydrates are known as either simple or complex carbohydrates. The simple carbohydrates are (i) three monosaccharides, which contain single units of either glucose ($C_6H_{12}O_6$), fructose, or galactose, and (ii) three disaccharides, which contain one of the three monosaccharides paired with a glucose unit.

Specifically, disaccharides consist of a glucose unit, paired with either fructose to yield sucrose, or glucose paired with lactose to yield galactose, or glucose paired with another glucose to produce maltose. The chemical reaction needed to form disaccharides is condensation, and the by-product of that reaction is the release of water. The opposite reaction needed to split disaccharides is hydrolysis, "hydro" (H_2O), "lysis" (split), by the addition of water. A glyosidic bond forms between the carbonyl and hydroxyl groups of two monosaccharides (in this case, glucose and fructose combine and form the disaccharide sucrose). An OH and a H (H_2O) are released with the formation of the disaccharide. The opposite reaction occurs when the bond is readily hydrolyzed (for example, by heat, acid, and enzymes, such as amylases, sucrase, or invertase) [1].

In addition to the simple carbohydrates of mono- and disaccharides, as well, carbohydrates include the polysaccharides. These are the complex carbohydrates, which consist of three constituents, i.e. three polysaccharides: glycogen, starch, and fiber. Glycogen is the glucose stored/found only in humans and animals (i.e. muscle, liver), not in plant material and not a source of dietary carbohydrate. Starches are the storage form of glucose for plants, grains, and legumes (beans, peas). Starches may be made of one or both of the two starch forms: amylose (i.e. glucose units arranged linearly with β-1,4 glycosidic linkages) and amylopectin (i.e. glucose units 1,4 linkages, with α-1,6 branching every 15 to 30 units). Fiber is found only in plant material, not animal, and unlike starch, it contains a 1,6 bond between glucose units rather than a 1,4 bond. The human enzymes can digest alpha starch bonds and cannot digest the beta fiber bonds of cellulose. Fiber exists as both soluble and insoluble fiber [2].

Soluble fiber dissolves in water to form a viscous, gel-like substance which is readily digested/fermented by bacteria in the colon. It lowers cholesterol, slows glucose absorption, and slows passage through the colon (preventing diarrhea). Foods such as fruits, oats, barley, legumes, some vegetable and plant fiber, and psyllium contain soluble fiber. Examples of soluble fiber are pectin, gum, mucilages, and some hemicelluloses. It aids in protecting against (i) heart disease by reducing cholesterol in the body (it is eliminated in the stool and is prevented for uptake into the body), and (ii) diabetes by reducing blood glucose levels. As well, blood pressure reduction (less hypertension), less risk of atherosclerosis, and less coronary heart disease are also apparent in association with good fiber intake. Soluble, viscous fiber provides a slow gastric emptying of food, and thus fiber intake proves to be a helpful strategy in weight loss [2].

Insoluble fiber is different from soluble fiber. This type of fiber increases the movement of material through the digestive tract and increases stool bulk. Insoluble fiber does not form gels. It increases stool bulk, softens stool, delays glucose absorption, and it speeds up passage through the gastrointestinal tract (thus, it prevents constipation). Insoluble fiber delays starch breakdown (good in the case of diabetes). These are included in the vegetables, wheat, and grains, for example, cellulose, lignin, and many hemi-celluloses. It assists in promoting "regularity" of the stools and is included in vegetables, whole wheat foods, bran, nuts, seeds, and the skin of some fruits and vegetables.

Fiber, carbohydrates that have not been digested in the small intestinal tract, is digested to a variable extent when it enters the large intestine. The bacterial flora metabolize these compounds anaerobically to carbon dioxide, methane, and short-chain fatty acids (acetate, propionate, and butyrate) which are rapidly absorbed and metabolized primarily by the colonocytes. Indigestible polysaccharides such as cellulose (which consists of glucose units linked by β-1,4 linkages) are part of dietary fiber that passes through the intestine without full digestion and absorption [2].

Fiber provides the human body with the "roughage" needed to sweep the intestine clean of the build-up of materials, and thus it serves a valuable purpose. Fiber helps with motility, which is the ability of the gastrointestinal tract to move. Fiber does not provide the same number of calories as other carbohydrates, and it is not processed in an identical fashion. The recommended intake of fiber ranges from 20 to 35 grams/day for women and men, respectively. Fiber promotes the health of the large intestine and protects against both constipation and diarrhea, as well as appendicitis, diverticulitis, hemorrhoids, and cancer such as colon cancer.

The milling technique for removing fiber from whole grain flour (which began in the 1890s) produces white flour. The health benefits of higher fiber intake lowered the risks of metabolic disorders, such as obesity and diabetes, and cardiovascular diseases, such as heart disease and high blood pressure. The processes of chewing coupled with enzyme action and enough digestive fluids change fiber. However, it is not by these actions, rather it is by the trillions of bacteria living in the large intestine, that the true transformation of fiber occurs.

Specifically, fiber aids in reducing sensations of hunger and the subsequent intake of food. However, it must be noted that too much fiber, consumed in a relatively short time, may lead to binding minerals, leading to nutrient deficiencies or diarrhea and bloating. The acceptable macronutrient distribution range (AMDR) for carbohydrate intake, according to the Institute of Medicine, is 45 to 65 g, indicating that 45–65% of daily calories should come from carbs. The AMDR for adults is 10 to 35% for protein and 20 to 35% for dietary fat. Fiber content remains similar in fresh and frozen, as well as heat-processed fruits and vegetables.

Glucose is absorbed through portal blood to the liver. Fructose and galactose are converted to glucose in the liver. The only sugar utilized by the body is the glucose. Most of it is taken by the liver to be stored as glycogen or oxidized by glycolysis for acetyl Co A and lipid synthesis. A minimal amount passes through systemic circulation to maintain the blood sugar level in fasting conditions (the fasting blood glucose level is 60–110 mg/dl) [3]. As a general rule, carbohydrate-containing foods rich in free glucose, oligosaccharides are easily digested and absorbed and have a relatively high glycemic response (increase in blood sugar level). Low-glycemic index foods (legumes, pearled barley, grains) are a good choice for chronic diseases including cancer and diabetes [4]. It has been suggested that cooking and food processing (degree of starch gelatinization, food form, particle size, and food structure) play an important role in determining the glycemic index foods. In summary, the human body benefits from the consumption of complex carbohydrates as well as simple carbohydrates.

51.2.2 Protein

Proteins are polypeptides (polymers of amino acids). Sources of dietary proteins include animals (milk, fish, and meat) and plants (cereals and beans). Protein as a biomolecule is found in DNA, RNA, glycoproteins, and glycolipids [5]. It is the building unit for skeletal muscles as well as being an integral part of different cell membrane receptors, ions channels, and hormonal transports. Proteins as a peripheral on integral spanning molecules in the cell membranes play a vital role in cell membrane fluidity and hence the signal-transduction pathways which are essential for cells' normal function and homeostasis [5]. The biological functions of protein are classified into transport functions (albumin that transports some drugs, calcium, bile pigments, and free fatty acids, and hemoglobin that transports oxygen, and transferrin that transports iron and lipoproteins), metabolic control (enzymes and hormones, e.g. insulin and glucagon), skeletal proteins (actin and myosin), immune function (i.e. immunoglobulins, epitopic determinants), blood clotting factors (i.e. fibrinogen, thromboplastin, and thrombin), essential components of cell membrane and receptors, structural functions (collagen, elastin, keratin, and rhodopsin) [6].

Protein is composed of amino acids, linked together by peptide linkages. The side group "R" is the amino acid component that varies from one amino acid to another**.** There is a variety of 22 possible amino acids, nine of which are called essential amino acids as the human body does not make them. Proteins exist as either animal- or plant-based and yield 4 calories per gram when broken down. The acceptable macronutrient distribution range (AMDR) for proteins is within 10 to 35% of the diet. In some severe cases of a protein energy malnutrition (PEM) disease, kwashiorkor may appear. Kidney disorders may result from overconsumption. It is predominantly proteins to which a person shows negative food allergy symptoms. Labeling for a food's eight major allergens is required in the U.S., as guided by the FDA.

The eight most common food allergies, accounting for 90% of food allergic reactions, are listed herein, yet over 160 foods can cause allergic reactions in people with food allergies. These foods are also the food sources from which numerous food ingredients, thus perhaps allergens, are derived (FDA). The eight foods are (i) milk, (ii) eggs, (iii) fish (e.g. bass, flounder, cod), (iv) crustacean shellfish (e.g. crab, lobster, shrimp), (v) tree nuts (e.g. almonds, walnuts, pecans), (vi) peanuts (peas, not nuts), (vii) wheat, and (viii) soybeans. Regarding food allergies, these eight foods, as well as any ingredient that contains protein derived from one or more of them, are referred to as "major food allergens." Knowledge continues to expand about the physiology of protein and amino acid metabolism in the mammalian organism and especially in relation to human protein and nutrition metabolism; accordingly, to help Americans avoid the health risks posed by food allergens, the U.S. Congress passed the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). Food labels must identify these allergens prominently on the ingredients label. Other countries, such as others in North America, outside of the U.S., report differently [7].

51.2.3 Fat

Lipids are important dietary constituents. They are present in the diet mainly in oils, butter, liver, brain, and egg yolk.

Dietary lipids are ingested in the form of triglycerides, cholesterol, phospholipids, and free fatty acids. Lipids are the major source of energy in the body; 1 gram of fat yields 9.3 kcal [8]. Also, oxidation of fat stores provides energy which is enough to allow several days of survival during total food deprivation, while glycogen oxidation gives energy enough to sustain the functions of the body for 24 hours of fasting. They serve as structural components of the cell membrane (phospholipids and glycolipids). They provide an adequate supply of essential fatty acids and fat-soluble vitamins (A, D, E, and K) [8]. In addition, lipids represent a source of steroid hormones and prostaglandins. Adipose cells are specialized for synthesis and storage of triglycerides in their cytoplasm (in fed state) and for their mobilization into fatty acids and glycerol that are transported to other tissues by blood (in fasting state) [9].

Fats consist of triglycerides, phospholipids, and sterol as triglycerides contain glycerol and three fatty acids, phospholipids contain glycerol, two fatty acids, and phosphorus, and have sterol rather than fatty acids in the structure. Fat content in food increases when it is cooked in a fat-soluble medium. Fats and water do not mix well in food without adequate emulsification [8].

51.2.4 Vitamins and Minerals

Micronutrients are the vitamins and minerals, and these are non-calorie-containing nutrients. These may exist in foods as naturally occurring nutrients, or as added nutrients through the process of either enrichment or fortification. Vitamins are the primary nutrient capable of being lost in the process of preserving foods. Vitamins are either water- or fat-soluble. Water-soluble vitamins include the B vitamins and vitamin C and are easily oxidized in steam. In order to maintain these water-soluble vitamins, the cooking water should be minimal, and product size kept large with less surface area than if cut small-sized. The four fat-soluble vitamins, A, D, E, and K, are not lost in external watery environments, but rather in external cooking fat. Minerals generally remain intact when food is preserved.

51.2.5 Water

Water is the sixth nutrient, and consists of hydrogen and oxygen. It is either added to foods or it is removed. Removal is either deliberate through the process of dehydration, or inadvertent. In fact, very low or high moisture may render a food unacceptable to the palate. An appropriate amount of water aids in retaining high-quality foods with nutritive value, taste, appearance, and textural quality.

51.2.6 Phytochemicals

Plants manufacture chemicals known as phytochemicals that have multiple functions. Some attract insects to encourage fertilization; others provide defenses against predators such as viruses and animals. Phytochemicals exhibit diversified physiological and pharmacologic effects. Active derivatives extracted from leaves, stems, roots, flowers, and fruits of plants may be classified into three main categories: (i) toxic and of no therapeutic use, such as pyrrolizidine alkaloids, nicotine, and hydrazine derivatives, (ii) toxic but useful for treatment of disease when used in controlled amounts or defined chemical conditions, such as morphine, digitalis, vinca alkaloids, (iii) chemo-preventive activity, compounds useful against diseases, such as atherosclerosis, cancer, and diverticular disease [10].

Medicinal plants have vast potential in the treatment of various ailments due to the presence of therapeutically important phytochemicals. Flavonoids are one of such groups, belonging to the superfamily of polyphenols, which have been investigated broadly for their medical properties. Fruits, vegetables, grains, wine, and teas, which are rich in flavonoids, seem to have the ability to prevent or even reverse human chronic diseases by numerous mechanisms including the primary prevention of oxidative stress [11].

Flavonoids and the other phenolic compounds are commonly known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl group. More than 8000 phenolic compounds as naturally occurring substances from plants have been reported [12]. It is very interesting to note that half of these phenolic compounds are flavonoids presenting as aglycone, glycosides, and methylated derivatives [12]. These phytochemical substances are presented in nutrients and herbal medicines; both flavonoids and many other phenolic components have been reported on regarding their effective antioxidant, anticancer, cardioprotective agents, anti-inflammation, and immune system-promoting properties, for skin protection from UV radiation, and as interesting candidate for pharmaceutical and medical applications [11, 12].

51.3 EFFECTS OF FOOD PRESERVATION METHODS ON NUTRIENTS

As far as possible, when food safety is considered, foods should be consumed raw and at the peak of freshness, in order to maximize nutrient intake. Follow food safety rules such as avoiding the consumption of uncooked meats, poultry, fish, and eggs. Cook for a shorter time if it is safe to do so (again, not usually meat, poultry, and fish). Minimize exposure to air, heat, cold, and water. Reuse cooking water containing water-soluble nutrients that have leached out during cooking. When possible, cut produce into large pieces rather than small, for preservation and nutrient retention in the cooking process. For comparison purposes, we will look at fresh foods first—fresh foods are generally consumed prior to postharvest nutrient degradation. This is optimal for maximum availability of nutrients. Cooking in water makes the water-soluble vitamin C from fresh fruits and vegetables susceptible to losses during cooking and thermal processing. Heat, light, and oxygen also cause nutrient losses. Minimal storage time and the correct temperature are crucial to the maintenance of vitamin C and overall nutritive value. Vitamin C content rapidly diminishes during greater than minimal storage, as illustrated in Table 51.1.

TABLE 51.1
Typical Maximum Nutrient Losses (as Compared to Raw Food)

Vitamins	Freeze	Dry	Cook	Cook + Drain	Reheat
Vitamin A	5%	50%	25%	35%	10%
Retinol equivalent	5%	50%	25%	35%	10%
Alpha carotene	5%	50%	25%	35%	10%
Beta carotene	5%	50%	25%	35%	10%
Beta cryptoxanthin	5%	50%	25%	35%	10%
Lycopene	5%	50%	25%	35%	10%
Lutein + zeaxanthin	5%	50%	25%	35%	10%
Vitamin C	30%	80%	50%	75%	50%
Thiamin	5%	30%	55%	70%	40%
Riboflavin	0%	10%	25%	45%	5%
Niacin	0%	10%	40%	55%	5%
Vitamin B_6	0%	10%	50%	65%	45%
Folate	5%	50%	70%	75%	30%
Food folate	5%	50%	70%	75%	30%
Folic acid	5%	50%	70%	75%	30%
Vitamin B_{12}	0%	0%	45%	50%	45%
Minerals	**Freeze**	**Dry**	**Cook**	**Cook + Drain**	**Reheat**
Calcium	5%	0%	20%	25%	0%
Iron	0%	0%	35%	40%	0%
Magnesium	0%	0%	25%	40%	0%
Phosphorus	0%	0%	25%	35%	0%
Potassium	10%	0%	30%	70%	0%
Sodium	0%	0%	25%	55%	0%
Zinc	0%	0%	25%	25%	0%
Copper	10%	0%	40%	45%	0%

Table 51.1 represents maximum nutrient losses in freezing, drying, cooking, cooking (and draining), and reheating, based on USDA data. The table compares the typical maximum nutrient losses for common food processing methods. This table is included as a general guide only. Actual losses will depend on many different factors, including the type of food and cooking time and temperature. For additional data on specific preparation methods, please see the USDA Table of Nutrient Retention Factors. Decidedly, there are additional, numerous ways in which foods can be preserved besides freezing, drying, cooking, and so forth [13].

The preservation of fresh foods is needed in order to prevent quality degradation, including nutrient losses. The end desired result of food preservation is food safety and a similarity to the fresh counterpart. Nutrients are lost during harvest to processing and during storage until foods are consumed. Preservation of nutritional value in fresh, frozen, and canned products is reported by Barrett. Some highlights of preservation of nutritional value in fresh, frozen, and canned products are reported by Barrett [21, 22].

"The USDA provides the most current data on nutrient retention. In this report 290 foods are referenced about their nutrient losses that are typical with common food processing methods such as freezing, drying, cooking and reheating." It explores "The specific factor representing the amount of the food component retained during the specified treatment."

51.3.1 Drying/Dehydration

Nutrient loss occurs immediately after the food item is cut and exposed to the surrounding air and dried. The vitamin A precursor beta-carotene and vitamin C are lost during drying. Commercial losses are 10–50% and 30–80% for these vitamins, respectively, and greater if preserved at home. Pretreatments may either save or cause loss of nutrients upon drying. For example, pre-treatment with sulfur dioxide (not used for organic produce) protects vitamins A and C yet causes a loss of vitamins B [14]. The nutritive value of freeze-dried foods was shown to be retained and higher than that of other drying methods. For example, with freeze drying, vitamins A and C level changes were not significantly different as compared to fresh. Freeze drying also shows a less negative effect on food shrinkage and taste as compared to the common dehydrating techniques. Potential nutrient losses in the dried green, leafy vegetables were observed. The beta-carotene is retained in the range of 20–69%, vitamin C up to 14%, and thiamin is retained in the range of 22–72% in the case of steam-blanching for 5 minutes followed by oven drying [15, 16]. Other diminished levels of nutrients such as polyphenols

were reported. [17, 18]. Upon rehydrating, and cooking with excessive heat, some water-soluble vitamin C and minerals may leach out, and be lost.

51.3.2 Heating. Mild (Blanching, Pasteurization)

Fewer nutrient losses occur through preservation techniques involving cold than through techniques involving heat [19]. Food preservation may involve mild techniques of heat treatment such as blanching or pasteurization. Even mild blanching, usually by steam or water, may result in a reduction of nutritive value via loss of some water-soluble vitamins (vitamin Bs [thiamin, riboflavin, and niacin of the B complex] and vitamin C). Fruit juices high in vitamin C, such as lemon, orange, or pineapple, ascorbic acid, or commercial products containing ascorbic or citric acid may be used for dipping new surfaces of cut fruits to prevent browning [20]. If added, there is an increase in nutritive value.

51.3.3 Heating (Canning)

As previously mentioned, the heat treatment of canning is classified as "severe," not mild. As a result, some water-soluble vitamins are destroyed. Canning is more deleterious to the structure and nutritive vitamin value of foods, such as fruit and vegetables, than if the food were untreated and eaten at the peak of freshness, soon after harvest. Canning exposes fruits and vegetables to high temperatures which degrade vitamin C and may cause leaching into the brine in the can. Vitamin C decreased by 10–90% during the canning of various vegetables, but there was little change in content during the storage of canned products [21]. The canned food processing treatment results in the loss of water-soluble nutrients such as vitamins C and B, and polyphenols, as these may leach out into the cooking water or canning medium. The actual cooking time and use of water of a canned product are less than those needed for a fresh or frozen product. This results in less vitamin C loss in cooking canned foods [21] As far as the B vitamins, thiamin and vitamin B6 specifically, which are very sensitive to heat and light, canning nutrient losses vary from 7 to 70%, depending upon the vegetable canned. Freezing and canning processes may relatively preserve nutrient value as compared to drying [22].

Heating may increase the presence of some antioxidant levels in fresh produce. This is true of the antioxidants beta-carotene and lycopene in tomatoes and carrots. A frozen vegetable company states the following: "Although freezing and canning may reduce the nutritive value of fruits and vegetables to a degree, they extend the length of time they are available. Once the products are processed, nutritive losses in the canned or frozen products are minimal if the products are stored and handled appropriately" [22].

Food processing may be achieved by heat, cold, or other means. The nutritive value of foods must be considered and maintained during food preservation [21–23]. Of the plethora of foods available for consumption, for example, fruits and vegetables, grains, dairy, meat, poultry, and fish, beverages, and so forth, many have been well-received worldwide. Food composition, including flavor, may be altered in the process of preservation. Preserving herbs (herbaceous, leafy part of plants) in cooking oil or vinegar aids in retaining/imparting flavor. Also, lactic fermentation compared with unfermented food may impart nutritive (and enzymatic) benefits of a more robust level. In food preservation, the nutrients must also be retained. More can be explored about the effects of processing on some nutritionally significant compounds in foods. Proper storage and timely consumption should occur for all consumers interested in healthy diets.

51.3.4 Refrigeration

Consumers may not detect that many fruits and vegetables rapidly lose their nutritional value when stored for more than a few days. After being purchased and stored in the refrigerator, some fruits and vegetables can lose as much as 50% of their vitamin C and other nutrients, depending on the temperature [23].

51.3.5 Freezing

Published studies comparing the nutritive value of fresh, frozen, and canned fruits and vegetables are limited. Noted more prevalently in studies are changes to color, texture, and flavor attributes. More inert, unchangeable nutrients such as minerals and fiber levels were similar in fresh, frozen, and canned fruit and vegetable products. These are not sensitive to the heat and subsequent degradation of food preservation. Sensitive to heat, light, oxygen, and pH, vitamins A, E, and lycopene may be lost in processing and preservation treatments. An advantage of cooking is that there is better bioavailability of some nutrients, and preservation may be better assured with some level of cooking foods (parboiling, and so forth). Cooking can both positively and negatively affect foods—a vegetable's color (pigment), texture (hemi-cellulose, starch, etc.), flavor (stronger or milder), and nutritive value (B vitamins, vitamin C, calcium, and so forth).

A more comprehensive discussion is based on a review of literature by Rickman et al., using the nutritional value of fresh, canned, and frozen fruits and vegetables [24, 25]. The conclusion regarding produce is that "Exclusive recommendations of fresh produce ignore the nutritional value of canned and frozen products and may conceal the sensitivity of fresh products to nutrient loss. "Although fresh-picked produce stored for a short time under optimal conditions and consumed raw will most likely provide maximal nutrition, the availability of such produce is limited by region and 'seasonality'."

Vitamin C losses during freezing were slightly lower as compared to canning. Losses were strongly influenced by the individual commodity, and the blanching and freezing methods utilized. If the storage temperature was well-maintained, the frozen fruit and vegetable products showed little change in nutrient content [24].

The short heating time, i.e. blanching pretreatment prior to freezing, inactivates enzymes and results in only a minor

TABLE 51.2
Factors That Remove Nutrients from Food

Nutrient	Heat	Air	Water	Fat
Vitamin A	X			X
Vitamin D				X
Vitamin E	X	X		X
Vitamin C	X	X	X	
Thiamin	X		X	
Riboflavin			X	
Vitamin B_6	X	X	X	
Folate	X	X		
Vitamin B_{12}	X		X	
Biotin			X	
Pantothenic acid	X			
Potassium			X	

loss of the foods' nutritive value. The various B vitamins were shown to lose 20–60% upon blanching and freezing [25]. Table 51.2 illustrates the typical maximum nutrient losses as compared to raw food and compares the typical maximum nutrient losses for common food processing methods. This table is included as a general guide only. Actual losses will depend on many different factors, including the type of food and cooking time and temperature. For additional data on specific preparation methods, please reference the USDA "Table of Nutrient Retention Factors." In it can be seen that the preservation processes that expose foods to high levels of heat, light, and/or oxygen cause the greatest nutrient loss [25].)

51.3.6 Pickling

The amount of fiber in fresh-pickled vegetables is usually similar to fiber in cooked vegetables. Fat-soluble vitamins, such as A, D, E, and K, are similar during pickling and when cooked. Korean researchers have found that kimchi (a spicy pickled cabbage), has up to twice as much of some B vitamins as compared to fresh cabbage. Certain vitamins are lost during pickling. For example, the heat necessary for canning pickled vegetables destroys much of the vitamin C. Light destroys riboflavin. The storage of pickled vegetables in a cellar or dark room serves to reduce exposure to light, and the subsequent destruction of riboflavin [26].

51.3.7 Radiation: Microwaving and Irradiation

It is reported that microwave heating inactivates vitamin B_{12}, which is found in animal products and fortified vegetarian products [27]. The study compared microwaving or steaming vegetables, such as cabbage, carrots, cauliflower, and spinach, to pressure cooking. It was found vegetables that were pressure cooked lost more insoluble fiber, which is good for gut health, than those that were microwaved or steamed. A key nutrient usually destroyed when cooking vegetables is vitamin C, a severe lack of which can lead to conditions like scurvy [28]. But microwaving accounts for greater nutrient losses than irradiating. This is because water-soluble nutrients are readily destroyed when they are heated, in microwaving [28].

51.4 CONCLUSION

Food provides the ideal mix of vitamins, minerals, and other nutrients. But the nutrients in foods begin to decrease as soon as the fruit or vegetable is picked and continue to decline until the food is eaten. The sooner you eat the food, the lower the chance of nutrient loss. Water-soluble vitamins, especially thiamin, folic acid, and vitamin C, can be destroyed during improper storage and excessive cooking. Heat, light, exposure to air, cooking in water, and alkalinity are all factors that can destroy vitamins. If food is not eaten within several days, freezing is the best method to retain nutrients. The handling, storage, and preservation of food often involve changes in nutritive value, most of which are undesirable. The freezing process, if properly conducted, is generally regarded as the best method of long-term food preservation when judged on the basis of retention of sensory attributes and nutrients. The freezing process is, however, not perfect, as is apparent from the fact that substantial amounts of the more labile nutrients can be lost. Vitamin losses during freezing preservation vary greatly depending on the food, the package, and the conditions of processing and storage. Losses of nutrients can result from physical separation (e.g. peeling and trimming during the pre-freezing period, or exudate loss during thawing), leaching (especially during blanching), or chemical degradation. The seriousness of these losses depends on the nutrient (whether it is abundant or meager in the average diet), and on the food item (whether it generally supplies a major or a minor amount of the nutrient in question).

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52 Packaging as a Preservation Technique

Mohammad Shafiur Rahman

CONTENTS

52.1 INTRODUCTION

Packaging has been with humans for thousands of years in one form or another. Packaging dates back to when people first started moving from place to place. Originally skins, leaves, and bark were used for food transport. Mesolithic humans used baskets and Neolithic humans used metal containers and discovered pottery. Four thousand years ago sealed pottery jars were used to protect against rodents, and in 1550 B.C. glass making was an important industry in Egypt. Tin plating iron became possible in A.D. 1200 and as steel replaced iron, this method became useful after A.D. 1600. In 1825 Oersted first extracted aluminum [1]. The Greek and Roman times saw the rise of pottery and the start of the use of glass. In the 1400s timber chests were first used, and after 1850 paper and glass started to be used substantially as processes were developed for mass production. Napoleon Bonaparte was involved in the invention of canning. More recently plastics were developed, particularly the first commercial plastics in the United States in 1935–42 [1].

One hundred years ago there was little use for packaging in the food industries. Now tremendous progress has been made in the development of diversified packaging materials, package design, packaging equipment, and environmental safety and sustainability. Over the last three decades, packaging has grown in volume and importance into one of the most significant areas of food production. This chapter provides an overview of food packaging.

52.2 PURPOSE OF PACKAGING

In addition to the direct approach to food preservation, such as drying and freezing, other measures such as packaging and quality management tools need to be implemented in the process to avoid contamination or recontamination. Although these measures are not preservation techniques, they can play important roles in producing high-quality and safe food [2]. Packaging performs five main functions: product containment, preservation and quality, presentation and convenience, protection, and provide storage history—the 5Ps.

52.2.1 Product Containment

The first function of packaging is its capability of containment. The primary purposes of packaging are containment and protection. It is self-explanatory; liquids, semiliquids, and powders, as well as bulk solids, cannot be marketed without suitable containers. According to the size of the package, different amounts of the product can be delivered

to consumers suiting their choice and convenience [3]. In certain circumstances, quantification is mandatory, as in the case of medical pills or capsules that are marketed individually in a blister-type package. Containment refers to holding goods in a form suitable for transport, whereas protection refers to safekeeping goods in a way that prevents significant quality deterioration.

52.2.2 Preservation by Maintaining Quality

The second function is to control the local environmental conditions to enhance storage life and safety. The main purpose of food packaging is to protect the product from the surroundings and to maintain the quality of the food throughout the product's shelf life. Packaging is one means of spreading product availability over time. Product shelf life is controlled by three factors: product characteristics, properties, and storage and distribution conditions of individual packages [4]. Reactions causing deterioration in foods include enzymatic, chemical, physical, and microbiological changes. Additional problems include insects, pests, and rodents.

52.2.2.1 Nutritional Quality

Packaging affects the nutritional quality of foods. Examples include peroxidation of polyunsaturated fats and destructive oxidation of nutrients such as ascorbic acid, tocopherols, vitamin A, folate, and riboflavin. Fatty acid peroxides are well established as causing health problems. As antioxidative nutrients such as vitamins C and E are lost, other food components become even more vulnerable to oxidation. Carotenoid pigments can also be oxidized, leading to loss of color as well as loss of their beneficial effects in the body. Lipid hydroperoxides can also result in the formation of aldehydes and other compounds with off-flavors. Such peroxides can act as free radicals, which, in turn, can damage other food components such as proteins [5].

Light induces photodegradation and causes loss of vitamins, especially riboflavin (which also acts as a photosensitizer), β-carotene and vitamin C; production or degradation of free amino acids; increases peroxide value; formation of sensory unpleasant volatile compounds (methional, aldehydes, and methyl ketones); and color changes [6]. Oxidative rancidity occurs when oxygen reacts with unsaturated fatty acids either spontaneously on exposure to air (autoxidation) or in the presence of light and sensitizers (photosensitized oxidation) such as chlorophyll or myoglobin. Among all other functions of packaging, the protection of foodstuffs against light plays a key role, particularly during storage, transport, and sales display. Visible light covers a wavelength range of 380 to 700 nm and ultraviolet (UV) light a range of 200 to 380 nm. Photodegradative processes induced by UV light should be given priority because of its high energy content, which is capable of splitting certain chemical bonds. Fluorescent light can initiate photooxidation in foods. In addition to light barriers, use of UV absorbers in the packaging material can decrease lipid oxidation [7].

52.2.2.2 Barrier Properties

Many packaged foods contain living systems in the form of active enzymes and the respiration process. Indeed, the benefits of controlled atmospheres with less oxygen and more carbon dioxide result in part from slowing the effects of these enzyme systems. The carbon dioxide is sometimes generated within the package by enzymatic action. If there is gas exchange across the packaging plastic, the benefits of the effect are diminished [5]. Ethylene affects the physiological processes of plants. As a plant hormone, ethylene regulates many aspects of growth development and senescence, and is physiologically active in trace amounts (<0.1 ppm). It is a natural product of plant metabolism and is produced by all tissues of higher plants and by some microorganisms. Moreover nonethylene and nonrespiratory organic volatiles may also have physiological and/or quality efficiency [8]. Thus packaging can also play a part either by absorbing ethylene (or other volatiles) or preferential transmission of this gas.

To achieve optimum packaging performance, it is important to know product characteristics, properties of the individual package, and storage and distribution conditions [9]. The knowledge of product characteristics and conditions of storage and distribution dictates the required barrier properties of the packaging materials used for a specific application. Barrier properties include permeability of gases (oxygen, carbon dioxide, nitrogen, ethylene, etc.), water vapor, aromas, and light. These are vital factors for maintaining the quality of food. However, packaging materials cannot be chosen solely on the basis of their barrier properties. Factors such as processability, mechanical properties (tensile strength, elongation, tear strength, puncture resistance, friction, burst strength, etc.), and chemical resistance and interaction with the product must also be taken into account [9]. Environmental factors, such as temperature, relative humidity, and light intensity to which the product is exposed during storage and distribution must also be taken into consideration when selecting packaging materials. Transportation damage may happen to the product due to the vibration and other stresses during transport. The extent of such damage can be reduced by proper packaging, such as introducing padding and support and by adjusting the distribution pattern according to the transported product [9].

Current packaging techniques perform more on the preservation by direct interaction with the product. Earlier food packaging materials used to provide only barrier and protective functions. Now various types of active substances can be incorporated into the packaging material to improve its functionality and give it new or extra functions. Such active packaging technologies are designed to extend the shelf life of food while maintaining nutritional quality and safety. Active packaging technologies involve interactions between the food, the packaging material, and the internal gaseous atmosphere [10]. In some cases, the new concept of active or life packaging material allows a one-way transfer of gases away from the product or the absorption of gases detrimental to the product.

Smart packaging with antimicrobials adds a dimension to safety [11]. The extra functions they provide include oxygen

scavenging (absorbing oxygen gas in the package and preventing rancidity, and are being developed as forms of sachets or polymer additives), antimicrobial activity, moisture scavenging, ethylene scavenging, and ethanol emitting [12]. Floros et al. [13] reviewed existing active packaging products and patents. Now it is possible to tailor the atmosphere to suit the needs of the food product inside the package. Indeed, the package itself can be the main regulator of atmospheric conditions within the headspace. The modified atmosphere could provide consumers benefits including quality retention, additive reduction, and fumigant elimination, and match today's busy lifestyle by providing fresh, ready-to-eat take-home meals.

52.2.2.3 Antimicrobial Packaging

Antimicrobial packaging is a promising form of active food packaging. When antimicrobial agents are incorporated into a polymer, the material limits or prevents microbial growth. This application could be used for foods effectively, not only in the form of films but also as containers and utensils. The incorporation of antimicrobial agents with polymeric packaging provides an economic and labor-free way to solve food surface contamination problems [14]. Food packaging materials may obtain antimicrobial activity by common antimicrobial substances, radiation, or gas emission/flushing. Radiation methods may include using radioactive materials, laser-excited materials, UV-exposed films, or far-infrared-emitting ceramic powders. Irradiation sterilization of food packaging materials is not yet permitted by the U.S. Food and Drug Administration (FDA) [12]. A variety of films can be made with antimicrobial activities and various degrees of clarity for use as food packaging. An extensive list of antimicrobial agents used in packaging materials is given by Han [12]. One of the new technologies expected to enhance plastic film barriers to gases and vapors is glass (silica)-coated plastics or the use of other ultrathin coatings such as diamond or other chemically treated surfaces [15]. The development of coated packaging materials, which absorb or block the ingress of taints during container transport of food to export markets, will be of particular benefit. Active and intelligent packaging is still largely a research field, but some manufacturers have already developed sachets of substances that can change the atmosphere inside the package, such as oxygen scavengers. Oxygen removal is not always easy. Oxygen removal can be mechanical, but air in the packaging materials or residual oxygen cannot be effectively removed in many cases. Conventional oxygen scavenging is too slow to retard the changes in many products. Oxygen scavenging systems can also be incorporated in a plastic package, thus forming an integral part of its structure. There are significant technical and commercial advantages in not having to insert sachets or attach labels to trays or bags. They can be activated just prior to use. The package can be manufactured and stored under standard conditions, then triggered to an activated state prior to filling. Inhibition of lipid oxidation in fish muscle can be done by packing antioxidant-incorporated polyethylene film [16]. Oxygen scavengers can also be added in the cap of a glass container.

Different foods require different packaging solutions. For example, the deterioration of product quality in bakery products is due to crystallization of starch granules, causing staling; moisture uptake, causing a reduction in crispness; mold growth, as bakery products typically have a high moisture content; rancidity from exposure of lipids to oxygen; and drying out of the interior (crumb) of a loaf of bread. The main forms of packaging for bakery products have distribution rather than protection as their primary objective. Product quality is ensured by specifying a short shelf life. For example, bread should be consumed as quickly as possible. It is difficult to specify a packaging material for bread that could meet the competing requirements of keeping the crust dry and the crumb moist. Furthermore, moisture movement from starch to protein within the crumb leads to texture changes as the bread ages. Since the shelf life is short, the goal of bread packaging is partially moisture control, but its main purpose is to allow the product to be distributed safely and hygienically. The material used should be inexpensive, as bread is a staple. Materials such as waxed paper, cellophane, and polyethylene are commonly used in bread packaging. Bread was one of the first mass products to be packaged in plastic bags, and it is the largest single domestic consumer product to go into plastics packaging. For many bakery products, even this level of packaging is excessive. Cakes and pastries are often distributed in cardboard boxes that are not airtight, made possible with a waxed paperboard support. Packages containing pies must have air holes, so that the package can "breath" moisture during heating or cooling, preventing crust uptake of moisture [1].

52.2.2.4 Edible Film

Edible antimicrobial films are a particularly promising development. Edible coatings can inhibit the growth of microflora themselves, and these films also offer the opportunity to provide high concentrations of antimicrobials at food surfaces. Edible films may also be used to overcome some of the difficulties in maintaining modified atmospheres around packaged ripening produce. The ratio of the permeability values of the film to carbon dioxide and oxygen can be crucial [15]. Enzyme-generated biofilms for packaging fresh food can be developed from cross-links of proteins [17]. The most important properties to be evaluated in an edible coating are its microbiological stability, adhesion, cohesion, wettability, solubility, transparency, mechanical sensory properties, and permeability to water vapor and gases [18]. Recent approaches of edible coatings are to develop active envelopes to include oil consumption reduction in deep-fat fried products, transport of bioactive compounds, and shelf life extension of highly perishable products [18].

52.2.3 Presentation and Convenience

The third function of packaging is presentation and convenience. In many cases, these are the most important factors for consumers.

52.2.3.1 Presentation

Food labels are intended by law to provide the information that consumers need in order to be able to make the necessary decisions about food purchases. It is important to display the product in an attractive manner to the potential buyer. A cleverly designed and beautifully produced packaging can help sell a product, which is an essential ingredient of an effective marketing campaign. The packaging helps in distinguishing products on the shelf, which is a trait especially important when marketing low-fat or nutritional products. Furthermore, packaging must address communication, legal, and commercial demands. For a package to be effective, it must present the product well and should do its own publicity. The protective packaging may have flaps that can be opened to give a ready-made display for the product, whereas some stores may remove the protective packaging to display the product directly on the shelves, leading to a preference for rectangular containers. The clarity (haze) and gloss optical characteristics are important in packaging presentation. Design based on marketing includes apparent size, attention-drawing by giving an impression of quality, and clear innovative readability of the packaging label [19].

52.2.3.2 Convenience

In many instances, consumers have limited time to shop, cook, and clean; and they prefer prepared or ready-to-eat meals that taste homemade [20]. Different types of packaging design could include various forms of conveniences, such as easy opening, microwaveable packaging, tamper-proofness, and smart barcodes.

52.2.3.2.1 Easy Opening

In many cases, packaging provides convenience to consumers, for example, paper cartons for milk or juice with an ease-open and easy-pour cap, and thus could also increase consumption. Semirigid packaging containers, such as stand-up flexible pouches and stick packs for unit portion sizes, are shifting toward thin, light, and flexible plastics. The closures of any rigid and semirigid package are key for a real competitive advantage. Zippers, slides, and pressure-sensitive adhesive flaps with different designs are being used [21].

Convenience takes different forms [22]: easy to hold, use, open, and close; handheld consumption, such as in a car or at a desk enabling multitasking; easy to prepare, cook, and reheat; and ready-to-eat microwaveable meals. Other conveniences could be smaller portions, reclosable, and tamper-proof options.

Value-added packaging allows in-package cooking and facilitates on-the-go consumption. Self-heating containers are also being developed. In a system described by Webb [23], an exothermic reaction takes place with crushed limestone and starts the heating process. Users would push a button on the bottom surface of the can, and the container fully heats within 5 minutes.

52.2.3.2.2 Microwaveable Packaging

Changes in society, such as diminishing population patterns, increasing average age, smaller families, and more leisure time, as well as improvements in the quality of life, standard of living, and general level of education may also demand specific functions from packaging [24]. Eating styles, such as ready-to-eat meals, snacks, and microwaveable ready meals have been changed over the years, which need innovation in packaging. For children, the packaging might represent innovation or fun [25].

Today's consumer wants to buy food that is ready-to-eat or needs a minimum of preparation, and is good value for money [26]. With microwave food preparation increasing, there is a need for the packaging industry to confront the particular problems in designing packages that deliver microwave products to the dinner table. Food processors can accelerate the usage of microwave ovens by designing products and packages that allow microwave heating or cooking to provide quality [27]. Three types of materials—transparent to microwave, reflective to microwave, and absorbent—can affect the cooking [28]. The transparent materials are nonmetallic substances such as ceramics that are coated or filled with microwave absorbent materials. The reflective category is composed of all devices that are metallic and absorb heat [27]. Aluminum is often used to selectively shield microwaves or redirect microwave energy from certain areas or locations of food. For example, portioned meals need to be heated at different rates, and sensitive portions need to shielded so that the entire meal can be heated more evenly [29]. In the case of frozen foods, such as lasagna, microwave energy needs to be redirected from the edges to the center. Absorbent materials are used to generate localized heating. For example, surface heating using absorbent materials could mimic browning and crisping. Absorbents (i.e., subsectors) are commonly available as flat pads, sleeves, and pouches with various patterns [28]. Arcing between foils needs to be prevented, which could be achieved by foil components that recede from the edge of the package to avoid arcing with the oven walls [30].

The critical design aspect of microwaveable ready-to-eat meals is the safe release of internal steam buildup in packages after microwaving. The build-up pressure could injure the consumer during opening. In extreme cases, pressure could expand and explode the package in the microwave oven (a hazardous situation), and at least a mess to be cleaned up. The internal pressure could be relieved by a weak heat seal that fractures from excess steam pressure during microwave heating, or the incorporation of shrink-film covered vent spots that melt or fail as a result of steam pressure. Films with laser scored or perforated weaknesses are being designed, which would fail and release the internal pressure inside the microwave. These microperforations or partial cuts remain intact during distribution and initial heating, and fail only above extreme pressure [21].

52.2.3.2.3 Tamper-Proof Packaging

Consumers want to tamper-evident closures to avoid packaging being opened unnoticed. In general, tamper-proof packaging makes products more difficult to open so there is clearly a need to balance safety with consumer accessibility [24]. The tamper-resistant package is to alert the consumer that tampering has taken place and to provide visible evidence of

tampering. In many cases, consumers are willing to pay more for tamper-resistant packaging. Tampering can be classified into four categories: two for type of tampering and two for location of tampering [31].

Casual tampering or grazing happens in the store. People sometimes open a product to taste or smell it. Or people attempt to change a price by switching caps. These people usually intend no harm. Malicious, surreptitious tampering usually occurs outside the store. The tampered package is then placed on store shelves. For normal routes of entry, the casual tamperer opens the package and recloses it using the cap or the tear strip or the tear out easy-open end. A malicious tamperer often finds uses an evasive route of entry and the package is reclosed by any means other than the cap or the tear strip. The intended route of entry and therefore the tamper-resistant feature is left undisturbed [31].

Holograms provide good security for labels and they can guard against forgery because they cannot be copied successfully and are difficult to duplicate. A generic self-adhesive hologram tape with tamper-evident properties may be used on various substrates to protect, secure, and authenticate products. Hologram tape can easily disclose whether cartons or boxes have been tampered with. Another tool is holographic shrink sleeves that are reverse printed and attached with tamper-evident holographic stripe inside the sleeves [22].

Covert taggants are unique, hard-to-replicate particles that can be added to inks, paper, films, and other materials, and they can be detected with a specialized reader. Beyond barcodes and optical authentication systems, covert taggants are used to include additional security features on applications ranging from passports to packaging. In the food industry, they are used on premium wines and spirits. Other high-tech covert taggant uses include surface-enhanced Raman scattering (SERS), a nanoscale phenomenon, to generate robust, unique, secure optical fingerprints. SERS materials provide a unique signal that can be detected using specialized readers, and therefore provide highly counterfeit-resistant security solutions for a broad range of applications including brand security [22].

Cases of extortion or sabotage have also been reported. In the mid-1970s, child-resistant packaging became an issue, leading to the development of childproof lids for poisonous products. Tamper-resistant refers to the ability of the packaging to resist tampering (or opening), for example, for child protection, whereas tamper-evident refers to the ability of the packaging to reveal that it has been opened.

52.2.3.2.4 Smart Barcodes

Radio-frequency identification (RFID) tags increase convenience and efficiency in supply chain management and traceability. They can be read via radio, meaning without any specific orientation and at greater distances than what is required for barcodes. Whereas a barcode indicates the type of item it is printed on, an RFID tag indicates not only the type of object it is attached to, but also a unique serial number, and thus can distinguish a given package from every other one in the world [22]. RFID uses radio waves to track items wirelessly. It makes use of tags or transponders (data carriers), readers (receivers), and computer systems (software, hardware, networking, and a database) [19]. The working principles of an RFID system are [32] (i) data stored in a tag are activated by a reader when an object with an embedded tag enters the electromagnetic zone of a reader, (ii) the data are transmitted to a reader for decoding, and (iii) the decoded data are transferred to a computer system for further processing.

2D bar codes are capable of being scanned by smartphones to deliver additional information to users. The ScanLife™ solution goes a step beyond the ability to gather data and analyze by using a camera phone into an all-in-one 2D bar code reader to quickly access websites for product pricing and more. This type of technology could also be used for marketing campaigns. SnapTags™ delivers interactive functionality and traceability to mass marketing by making any logo a gateway to mobile marketing [22].

52.2.3.2.5 Design Simplicity

Packaging design has a strong impact on the point-of-purchase decision. Favier et al. [33] investigated simplicity/complexity through a multidisciplinary approach by mobilizing the fields of semiotics, art history, and marketing. They identified that simplicity/complexity of a package design has a significant impact on brand perception. Simplicity is associated with modernity, reliability, authenticity, success, and sobriety; and complexity with seniority, joy, imagination, charm, femininity, and sophistication.

52.2.4 Protection during Distribution and Processing

The fourth function of packaging is to protect the product during transit to the consumer. Packaging is part of the distribution process necessary to deliver goods to the consumer and facilitate handling and transportation. It also has affected international trade by making shipping of food products possible, allowing seasonal products to be more accessible out of season. Packaging can handle challenges in the food distribution chain, such as heat, humidity, and dew. It is important to aware of the distribution challenges when designing packages.

In the case of prepacked products, they should have the ability to withstand the severity or type of process conditions, such as flexible packaging during canning, and microwavable, ovenable, and retortable foods. Irradiated foods are usually prepacked prior to treatment by ionizing radiation, which prevents recontamination. Packaging materials are also exposed to radiation during treatment, though in this instance it can lead to radiation-induced degradation of the packaging material, followed by interaction between the material and food product [24].

Protective packaging is a term applied to packaging that is primarily designed to protect the goods, rather than for appearance or presentation. Generally protective packaging is applied to the outer containers used for transporting goods from the manufacturer to the point of sale, and filling materials inside the outer containers, e.g., nylon barrier-sealed

bubble packaging (computer parts), urethane expanding foam, polyethylene (PE) foam package "cushions," and polystyrene (PS) loose-fill packaging. The most widely used protective package is the outer carton. All packaging is protective as one of its primary functions, so it is more accurate to call this transport packaging or tertiary packaging (on the basis of the primary packaging being in contact with the product, secondary for grouping units together for single purchase, and tertiary being for grouping secondary packaging for convenience distribution). A pallet is the frame base for carrying the transport packs [1]. The primary packages are put into cartons and the sealed cartons are transported through specialized conveyors, allowing products from different processing lines and sorting onto individual product pallets.

52.2.5 Provide Storage History

A time–temperature indicator (TTI) is effective for predicting microbial concentrations and other parameters of food quality during shipping and storage. It helps ensure proper handling and provides a gauge of product quality (i.e., microbial conditions or ripening status) for sensitive products in which temperature control is imperative to efficacy and/or safety. TTIs are tags that can be applied to individual packages or shipping cartons to visually indicate whether a product has been exposed to time and temperature conditions that adversely affect the product quality. TTIs could be used in chilled foods to identify temperature abuse during storage, display, and distribution.

According to the response mechanisms, TTIs can be divided into three groups: (i) biological, (ii) chemical, and (iii) physical systems. One of them is the use of enzyme-based TTIs to monitor and predict shelf life of products. The tags are available in a one-dot version and a three-dot version with the three dots changing color at different rates. The change of color of the dot indicates the exposed time and temperature of the product [34].

There is considerable potential for the use of TTIs in the food distribution chain, but there are two issues to be considered. One is economics. When using a TTI for a relatively low-cost product, such as lettuce, the indicator also has to be relatively low in cost. This should be considered or addressed by the manufacturer of the indicator. The other issue is knowledge of the food product. The food processor must know the degradation kinetics of the product—how the quality characteristics of the product are changing with time and temperature exposure—in order to select the indicator that matches it [34].

The intelligent ripeness indicator (such as RipeSense™) responds to the aroma released as fruit ripens, giving consumers a better way to determine the ripeness of fruits before opening the pack, and thus provide optimum quality when consumed and reduce waste [22]. The gas concentration indicators in a pack could create tremendous applications, especially in modified atmosphere packaging. The most common oxygen indicator is pink when the ambient oxygen concentration is $\leq$0.1%, then turning blue when the oxygen concentration is $\geq$0.5%. The presence of oxygen is indicated in 5 minutes or less, although the change from blue to pink may take 3 hours or more. "Intelligent ink" has developed by using light-sensitive nanoparticles, such as titania (TiO_2) that can only detect oxygen [35]. The ink is blue in air and ambient room light, but the color changes to white if irradiated with a pulse of UV light. The color can revert to blue under normal room light. In an oxygen-free atmosphere, the ink remains colorless after the UV pulse. This type of ink could be inexpensive and could also be used to indicate if the original modified atmosphere inside a package has changed. Similar novel carbon dioxide intelligent pigment can be incorporated into a thermoplastic polymer to create a long-lived, carbon-dioxide-sensitive plastic film [36].

52.3 IDEAL PACKAGING

There is no such thing as the ideal packaging. Packaging should be such that we could come close to the ideal. The criteria of ideal packaging are [1]

- Zero toxicity
- High product visibility
- Strong marketing appeal
- Ability of moisture and gas control
- Stable performance over a large temperature range
- Low cost and availability
- Suitable mechanical strength (i.e., strength in compression, wear, and puncture characteristics
- Easy machine handling and suitable friction coefficient
- Closure characteristics, such as opening, sealing and resealing, and pouring
- Ability to include proper labeling
- Resistance of migration or leaching from package
- Protection from loss of flavor and odor,
- Controlled transmission of required or unwanted gases

52.4 TYPES OF PACKAGING MATERIALS

From skins, leaves, and bark, tremendous progress has been made in the development of diversified packaging materials and in packaging equipment. In general, packaging materials may be grouped into rigid and flexible structures. Plastic film, foil, paper, and textiles are flexible materials, whereas wood, glass, metals, and hard plastics are examples of rigid materials.

52.5 ENVIRONMENTAL ISSUES

Recently, ecological concerns about packaging have emerged. This means that packaging has to satisfy the required physical, chemical, and biological criteria during their life cycle as packaging (active protection function); and once the original function has been fulfilled, the packaging should decay without polluting the environment (passive protection function).

The presence of plastics in the habitat of wildlife on both land and sea has created issues, which are being vigorously exploited by the environmental lobby to demand solutions from the plastics industry [37]. Landfills are an important outlet for the disposal of packaging waste. Currently, modern facilities are designed to address two environmental concerns [38]. First, is to control the leachate (toxic polluting compounds) to the ground and surface water, and second is the control and use of landfill gases (carbon dioxide, methane, small amounts of nitrogen and oxygen, and a wide range of other gases). These gases are produced during biodegradation of waste from bacterial action, and these may be toxic or explosive. The composition of the mixture depends on the composition, temperature, moisture content, and age of the waste. Most modern landfills use a gas capture technology where the landfill gas is either fired to convert methane into carbon dioxide, or collected and used as a substitute fuel or to generate energy (i.e., energy recovery) [38].

Overall, environmental safety is demanded from packaging material disposal, thus the industry needs to develop easily reusable, recyclable, disposable, or environment-friendly packaging. It is also termed as sustainable, eco-friendly, environmentally-friendly, and green packaging [39]. In 2005, the U.S.-based Sustainable Packaging Coalition defined sustainable packaging by listing eight business and environmental criteria related to the life cycle of packaging [39]. Robertson [39] explained optimum packaging considering "useful" and "wasteful" and both "under-packaging" and "over-packaging" can have an environmental impact at different stages of the life cycle. Although under-packaging is less expensive as compared to over-packaging, environmental impacts are higher if packaging does not provide protection and spoilage occurs [40]. Food package functionality remains paramount; if the package does not function effectively and economically, all efforts are superfluous. Sustainability is collateral to food delivery [41].

Russell [42] emphasized that the whole value chain has a responsibility toward sustainability, including recycling and biodegradability of packaging. The overall resource efficiency of the supply chain should be the main priority. Heller et al. [43] defined a "Food-to-Packaging (FTP)" ratio for explaining the environmental impact. It is defined as greenhouse gas (GHG) emissions or cumulative energy demand for agricultural production and food processing divided by GHG for packaging material production based on a kilogram of food consumed. They reported FTP ratios for GHG emissions ranging from 0.06 for wine to 780 for beef. The high FTP for the foods such as cereals, dairy, seafood, and meats suggest greater opportunity for net impact reductions through packaging-based food waste reduction. Robertson [39] also pointed that the focus on recycling rather than overall sustainability also risks distorting outcomes.

52.5.1 Reduce

An important consideration could be the reduction of the amount of packaging used for foods. In many cases, the efficient design for preservation and the distribution chain could reduce the amount of excessive packaging required for food. It is important to use packaging in the optimum level in addition to only reuse and recycle. For example, the main purpose of packaging for bakery products have distribution rather than protection as their primary objective. Product quality is ensured by specifying short shelf life since bread should be consumed as quickly as possible. Since the shelf life is short, the goal of bread packaging is partially moisture control, but its main purpose is to allow the product to be distributed safely and hygienically. The excessive use of packaging could be avoided if the purpose of the packaging is known.

For many bakery products, even this level of packaging is excessive. Cakes and pastries are often distributed in cardboard boxes that are not airtight but made possible with a waxed paperboard support. Packages containing pies must have air holes, so that the packages can "breath" moisture during heating or cooling, preventing crust uptake of moisture.

There is a constant effort in the packaging industry to reduce the amount of material used for packaging. Glass containers now are on average 30% lighter than in 1980, the weight of cans now approximately 40% less than in 1970, and 2 liter polyethylene terephthalate (PET) soft-drink bottles are 25% lighter than in 1977 [38].

52.5.2 Reuse

Reuse refers to the use of the same material again and again ad infinitum or for an indefinite time. Reuse is the application of a structure for an extended period of time or until it wears out [44]. Some packages, such as glass bottles and jars, can return to the original supply chain and be reused [38]. Sand [45] pointed out that the "milk bottle approach" (i.e., reuse) is a diversion when sustainability of packaging is considered and painful to think about since these resources are much needed to combat climate change. She mentioned that reusable packaging may resonate with consumers, but it is not environmentally or economically sustainable. The life cycle assessment (LCA) and economics are far from favorable for a system that delivers and collects packages from scattered consumer residences and returns these to manufacturers far away for refilling. LCA may be more viable if returnable packaging is aligned with existing systems of garbage and recycling collection. It would be one step forward if integrated collection and consumer sorting of compostable, recyclable, reusable, landfillable, and incinerable packaging are considered.

52.5.3 Recycle

Recycle means to recover and reprocess something into the same or similar product or something useful so that the molecules are not lost in space. Recovering is the act of identifying, finding, and retrieving materials that could be returned to the sender for eventual recycling [44]. The present and future focus is to use materials that can be either recycled or burned without producing noxious fumes, and use of printers' ink without containing heavy metals or biodegradable inks [22, 46].

Contamination is the main concern in the case of recycling and reuse of post-consumer plastics. One of the many difficulties with recycling is that there is no control over how the consumer uses the container (e.g., for pesticides, chemicals), and the container may become contaminated. The ink on labels is also difficult to remove during recycling [1]. In the case of recycling, lamination could be utilized whereby the food contact surface is a virgin layer placed over the recycled material means the virgin layer free of contaminants acts as a functional barrier. Other solutions for recycling are incineration, composting, and environmental degradation. However, this needs to include biodegradable materials. In many cases, the energy requirement during recycling is also an important factor. PET bottles are 25% more energy-efficient than glass and 65% more efficient than aluminum and have less impact on resources at all levels of possible recycling [1]. In many cases, collection costs can be far greater than the potential market of these materials. Auditing recycling can provide an ounce of prevention that can help in avoiding the complications of improper disposal and the resulting liabilities.

52.5.4 Consumers' Attitudes toward Green Packaging

Green packaging has received increasing attention in the logistics industry due to the growing popularity of environmental protection, sustainability, and eco-friendliness [47]. The preference for green packaging varies [48]. Hao et al. [47] identified four principal factors affecting consumers' willingness to pay: environment, green packaging quality, commodity, and packaging price. Even though the majority of consumers have insufficient knowledge regarding green packaging, they have a fairly strong willingness to pay for it. Additionally, consumers would like to attach greater importance to the practicalities of green packaging, such as convenience, reusability, and protective capability [49]. Considering 25 variables related to green packaging, six factorial groups were identified from "theory of consumption values" and "customer value creation framework" in relation to uniqueness to green packaging and influenced buyers' willingness to pay a price premium [50].

The actual environmental impacts (by altering packaging materials) provide other benefits, such as perceived taste and quality in addition to the sustainability perceptions. In addition, consumers' sustainability assessments are highly influenced by mere graphical packaging cues, which have no obvious actual sustainability consequences [49]. In Germany, France, and United States, it was observed that consumers focus predominantly on end-of-life attributes of packaging, although they value recyclability, reusability, and biodegradability differently. There are a number of discrepancies between consumer perception and the environmental impact of different packaging options. It was suggested to feature those eco-advantages that pertain to the post-use phase and to apply different packaging strategies across countries [51]. The dimensions of sustainability have an effect on the selection of the optimal design [52].

52.6 CONCLUSION

In the future, industry and research should aim to satisfy most criteria mentioned earlier in the list of ideal packaging. The choice of packaging is always a compromise between the desired objectives. The quality, safety, freshness, and convenience expected to be the future target in the development of food packaging. Future packaging trends are to develop packages with the following characteristics: opening ease, smaller portions, safe to consumers, environmental packaging, bioactive packaging, biodegradable packaging, edible packaging, tamper-proof methods, and reclosable packaging. Oxygen scavenging technology is merely in its infancy and it has the potential of becoming one of the most revolutionary advances in the packaging industry. The packaging should also give lifestyle benefits. Packaging plays an indispensable role in modern society, for without it many products could not reach consumers in sound condition. In all cases, the amount of packaging used should be optimum, thus avoiding overpackaging. Collection and sorting are issues in reusable packaging, and the economic evaluation may not support then. Better management of product shelf life can reduce excessive packaging. Efficient distribution systems also need to be developed for optimum packaging. The whole value chain needs to be considered when evaluating green packaging including sustainability, recycling, and biodegradability. There is a need to educate the consumer for understanding all aspects of food packaging, and making them a partner for future development. This could help in making the progress smooth and steady to achieve the target of safe packaging technology for consumers.

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53 Types of Packaging Materials Used for Foods

Robert H. Driscoll and Mohammad Shafiur Rahman

CONTENTS

53.1 INTRODUCTION

Originating from natural materials such as skins, leaves, and bark, tremendous progress has been made in the development of diversified packaging materials and packaging equipment. Packaging materials are commonly grouped into rigid and flexible structures. Plastic film, foil, paper, and textiles are flexible materials, whereas wood, glass, metals, and hard plastics are examples of rigid materials.

53.2 PLASTICS

Polymers are the fastest-growing group of materials in food packaging. The first plastic materials used for flexible packaging entered commercial production in 1939, just as World War II started, but the main development took place in the middle of the 1950s [1]. Their foremost advantages are their wide diversity and extremely broad spectrum of properties. Plastics are relatively cheap, light, easily processed and shaped, and easy to seal. The density of most plastics is on par with that of paper or a shade higher, but less than half of the density of glass or aluminum or about one-eighth of the density of steel. Plastics do not shatter like glass or buckle like metals [1]. However, two major drawbacks of plastics are their permeability to gases and vapors, and the possibility of their interacting with the product [2]. Polymers (or, as they are commonly called, plastics) are compounds of very high molecular weight. They are constructed of many repeating units or building blocks, combined together via a chemical reaction. These building blocks, called mers or monomers, are gases or liquids at room temperature and pressure, whereas polymers are normally solids under these conditions. Polymers can be either natural (familiar examples are starch, proteins, and rubber) or synthetic, the latter being those used in packaging. Reviews on types of plastic packaging materials are given by Driscoll and Paterson [3], Stollman et al. [4], and Miltz [2]. Other components in plastics are residual monomer and oligomers, additives such as heat and light stabilizers, antioxidants, plasticizers, and UV absorbers, as well as processing aids such as lubricants, slip agents, and antistatic agents.

Since no single film can satisfy all packaging requirements, plastic films may be combined by lamination or coextrusion. Lamination is a technique for bonding films together to give a film the properties of both constituents. By combining the qualities of choice from the raw material films, a laminate can be tailor-made for its particular application. Each layer in the resulting laminate may exhibit different properties from its free state, such as mutual layer reinforcement in which cracks in a brittle layer are prevented from propagating by high elongation (elastic) layers. For package sterilization, the material of choice is polypropylene, which is used as the outer and inner plies of the laminate with polyvinylidene chloride as the middle layer to provide an oxygen barrier. Intermediate between these main functional layers will be other plies to contribute appropriate bulk and strength. Polypropylene has the additional advantage that it can be heat sealed [5].

53.2.1 Background

The first type of packaging material to be discussed is plastic, which is technologically a complex class of materials. Packaging materials may be grouped into rigid and flexible structures. Plastic film, foil, paper, and textiles are flexible materials, whereas wood, glass, metals, and hard plastics are examples of rigid materials. The volume of plastics produced each year now exceeds the amount of steel consumed, and practically none of it is recycled. Plastics now account for about 25% of household waste, although less than 20% of the containers we use are plastic. Half of these are used for milk and various carbonated drinks. However, using other forms of packaging would double packaging costs, quadruple the amount of waste products, would take more energy to produce, and reduce the number of new jobs per year created by the growing packaging industry (e.g., 2500 in Australia). There is increasing recognition of the need to recycle. The main difficulties are the separation of plastics (manual labor) and the purity of the final product (energy problems).

Because of their lower unit cost and lower energy consumption during manufacture, plastics have tended to replace the traditional packaging materials—glass, paper, and metals—in situations where high barrier properties are not required by the product. Although no ideal barrier plastic exists, developments in laminates and copolymers are slowly reducing the competitive edge of glass and metal containers. The historical developments of plastics are presented in Table 53.1. Since 1953, a range of other important plastics have been developed. The definitions of terms are also presented in Table 53.2.

53.2.2 Manufacturing of Plastic

Polyethylene is a polymer that is produced from oil or coal by the extraction of ethylene gas. Ethylene (C_2H_4) is a monomer, a molecule that can combine with itself through breaking its carbon–carbon double bond to produce long-chain macromolecules that are highly inert to the environment. Many other monomers are derived from ethylene.

The chain of mers can twist and kink around the C–C bonds so that the resulting macromolecule can vary from a straight line to a sphere. The degree of linearity has a large effect on the resulting plastic properties. As the polymer is cooled after

TABLE 53.1
Historical Development of Plastics

Year	Plastic Type
1843	Malayan gutta-percha, a shellac molding material, was the first seminatural plastic.
1870	Searching for a substitute for ivory for constructing billiard balls, Hyatt made pyroxylin from cotton and nitric acid and then reacted this with camphor, producing celluloid.
1909	Leo Baekeland reacted phenol with formaldehyde with a catalyst, hexemethyleneteramine, under pressure (to stop foaming), producing the first synthetic resin, called bakelite.
1919	Casein was developed as a film.
1927	Cellulose acetate and polyvinyl chloride were developed.
1935	ICI reacted ethylene under high pressure with trace O_2, giving LDPE, which suited the newly developed technique of blow-molding.
1953	Karl Zeigler produced HDPE from ethylene using the catalysts titanium tetrachloride and triethyl aluminum.

melting or manufacture, the chains will link to each other, either by van der Waals forces, by ionic attraction, or by cross-link bonding. If the macromolecules are straight and regular they will fit well together, giving higher density and crystallinity.

The kinked and twisted molecules are stretchable, sometimes to many times their unstretched length, so the plastic can be *oriented*. Branching can occur along the chains but is relatively rare, requiring reaction to occur at a C–H bond. Higher temperatures permit more of these random branching reactions to occur. Branching reduces crystallinity by preventing ordered molecular arrangements. Most polymers are from difunctional monomers; a few are tri- or multifunctional, leading to three-dimensional configurations instead of long chains. These structures are more rigid at high temperatures.

The plastics are divided into two groups: thermoplastic and thermoset. Thermoplastic means that the plastic may be heated and cooled without losing its structure, while thermoset plastics, once cooled, cannot be reheated without breakdown of the macromolecule. When a thermoplastic material is heated, it becomes pliable and can be shaped as required. Linear polymers tend to be thermoplastic, whereas cross-linked polymers are thermosetting. Cross-linking occurs when atoms join across polymers, e.g., sulfur, which is used to cross-link isoprene to give us the rubber used in car tires, or oxygen, which causes aging in the same rubber by cross-linking and so reducing the rubber flexibility. The structure of natural rubber (isoprene) is leaving out irrelevant hydrogen atoms.

The C after the double bond can point up or down, leading to isomers (isoprene and gutta-percha) with different properties. By analogy with isomers, the basic units can also combine in different orientations, leading to isotactic polymers with ordered structures, which can more easily be crystalline, and atactic structures, where the orientation continuously varies along the chains, preventing crystallinity. For example, polyethylene (PE) varies from atactic PE with a density of 900 kg/m^3 to crystalline PE with a density of 1000 kg/m^3. Common forms of PE are low-density PE (LDPE, 920 kg/m^3), linear low-density PE (LLDPE), medium-density PE (MDPE), and high-density PE (HDPE, 960 kg/m^3).

Bonds can be broken in a number of ways (degradation of the polymer). A C–C bond has an energy of 6.1×10^{-19} J. Ultraviolet (UV) light at 300 nm has an energy of 6.1×10^{-19} J/photon, so is close to the same energy. Visible light at around 600 nm has about half as much energy. If thermal or strain energy is also present, then visible light will also degrade plastic (but more slowly than UV).

Glass temperature refers to the temperature at which individual molecular energy is too low to allow the macromolecules to slide past each other. The melt temperature is the transition temperature from a solid to a free liquid and is higher than the glass temperature. Below the glass temperature, the plastic is brittle, so some plastics cannot be used for freezing, but between the two temperatures, the plastic can be shaped and bent more easily. A plasticizer acts by reducing the average molecular size, acting as a lubricant between the large macromolecules so that they slide over each other. Methods of polymerization are given in Table 53.3.

TABLE 53.2
Definitions of Terms

Terminology	Definition
Mer	A unit, e.g., a basic unit in a chemical structure polymer: many units
Plastic	Deforms, can be shaped by a shear force in excess of a plastic yield minimum
Thermoplastic	Shaped by reducing the yield stress by heat softening
Thermosetting	Initially shaped by heat, but shape is then set permanently crystalline: regular, periodic shape
Amorphous	Opposite to crystalline, random shape
Isotactic	Possessing a preferred orientation of molecules (same feel) atactic: molecules in structure are randomly oriented (no feel)

TABLE 53.3
Methods of Polymerizing

1. Addition: unsaturated compounds open their double bonds under heat and pressure. The macromolecule forms in three stages: initiation, propagation, and termination. Initiation is the initial rupture of the C=C bond to produce energetic combination sites. In propagation, further monomers react with a growing chain. In termination, two growing chains meet each other and combine, preventing any further reaction. For high molecular weights, this should be delayed as long as possible.
2. Condensation: after initiation, molecules combine, releasing low molecular weight compounds such as water.
3. Ionic (either anionic or cationic polymerization): initiation occurs through the introduction of ionic compounds, which become integrated into the polymer, producing a class of plastics called ionomers (surlyn).

Ethylene is the main monomer used in the production of plastics (CH_2=CH_2). The hydrogen atoms can be replaced by halides (chlorine, fluorine, and bromine), phenyl, or other such groups to produce further monomers, all containing the central ethylene double bond. These polymers are generally thermoplastic and are called polyolefins.

Large chemical companies usually manufacture plastic in the form of small plastic beads or resins. These granules as bulk are transported to factories for manufacture into either rigid or flexible materials. In either case, the resin is fed into the hopper of an extruder, which brings the resin temperature to above its plastic melting point but below its degradation temperature. It is then ready to be shaped by passing through a die plate, containing die inserts that shape the product as required.

53.2.3 Construction of Rigid Plastics

53.2.3.1 Blow Molding

Many plastics are shaped using the process called blow molding. This is a process for producing hollow parts in one operation and is primarily used to make plastic bottles. A hot plastic tube, called a parison, is inflated against a cold mold. The final shape will have a thin neck, ideal for many packaging applications. The parison is produced by extruding the plastic at high temperature and pressure. Variations on blow molding include coextrusion blow molding, injection blow molding, and injection stretch molding. Labeling can also be done in the mold.

53.2.3.2 Injection Molding

Injection molding involves the direct injection of molten plastic into a shaped space between two dies. The dies separate after the plastic cools to allow the final shape to fall out. This is used for making solid plastic objects such as caps, closures, and plastic toys.

53.2.3.3 Thermoforming

Many plastic containers are formed by thermoforming. This involves heating a plastic sheet until it softens and then shaping it by stamping it between two cooled molds. A growing requirement of molded plastics is that they be microwaveable, i.e., have a softening point well above 100°C. They must also be shelf-stable. Plastics are generally slow to degrade under ambient conditions, but they may discolor or become brittle.

53.2.4 Construction of Plastic Sheets and Films

Approximately 10 different polymers account for the majority of packaging films. In general, a film is defined as a layer of a thickness of less than 0.010 inch (0.25 mm). Thicker plastic is referred to as a sheet. Films may be used as liners, wraps, or overwraps, depending on how they are applied to the product. Liners are plastic films applied to the inside of the container, often to protect the container from the product, such as plastic linings sprayed into the inside of metal drums or cans. Wraps are applied directly to the product, usually by making a tube from the film, sealing one end, filling the tube with product, then sealing the top end. An overwrap is applied around the carton, often heat shrunk onto the carton to provide barrier protection.

Films may also be used for bags, envelopes, and pouches. A common method of closing plastic bags is with plastic clips that can also double as price tags, as in bread packaging. Envelopes are bags sealed on three sides, with the last side folded over and sealed after filling with product, used mainly for flat items. Pouches are constructed and filled on the processing line automatically. A typical pouch is a complex laminate, possibly of oriented polypropylene (outside), LDPE, aluminum foil, adhesive, and a copolymer film on the inside.

Films are manufactured using an extruder. The plastic resin granules and other ingredients are fed into the extruder, compressed, and heated until the plastic flows out of the die. The two main die shapes are slit circular and slit rectangular. The extruded plastic may then be stretched while still hot and flexible.

53.2.4.1 Monoaxially

Die is designed to give a wide sheet of plastic film. This can then be stretched in the machine direction by up to 80 times its original length, which has the effect of thinning the sheet and orienting the molecular structure. The result is that the plastic can be stretched easily in one direction after cooling, but not in the other. The thickness of the film is checked, the film cooled, and then wound onto the mill roll.

53.2.4.2 Biaxially

Jaws and rollers are used to stretch the film apart in both directions at once (Tenter process). The molten film is poured onto a casting wheel and then through a series of rollers, which stretch the film in the machine direction. The film then passes through the Tenter section oven, where jaws are used to grip the softened plastic and pull it apart in the transverse direction. The result is a stiff plastic. A second method is called the bubble method, where the plastic is extruded as a continuous cylinder, and a bubble of air is blown up the center of the cylinder, forcing biaxial expansion. As the plastic heat-sets, it will shrink slightly before assuming a fixed shape.

The plastic may then be coated prior to the mill roll in several ways to enhance its properties. A common example is coating a plastic that is difficult to print with a printable plastic on one or both sides. Coating will be discussed in more detail later.

Another common coating produces metallized plastic. For this technique, the plastic passes quickly through an evacuated chamber in which aluminum is vaporized. This deposits a metal layer several molecules thick (~200 Å), resulting in a metallized appearance. This material is often used to replace foils as it is cheaper but does not have the same barrier properties as foil (although there will be some improvement over untreated film). The metal coat also substantially reduces UV light reaching the product. Metallized films are a common base for eye-catching graphics. However, the metal does not attach firmly to the plastic and may be scraped off (a surface

lacquer prevents this), and the plastic is not sealable on the metallized side. Metallized plastics also suffer from metal oxidation, affecting print adhesion, giving a maximum effective lifetime of about 3 months.

The film may also be pearlized, a technique that distributes fine air bubbles throughout the plastic (or cavitating the surface) resulting in a pearly white opaque appearance. This is useful for preventing "show-through," the wet appearance where fats directly contact the plastic, and a particular problem with chocolate wrapping. Pearlized plastics also tear more easily. The ideal film should have good barrier properties; be chemically stable, temperature resistant, and heat-sealable; resist grease; be strong; not allow migration; have good transparency and gloss; be printable; and be inexpensive to make. Since no plastic will satisfy all of these requirements, the actual plastic chosen for a particular function will be a compromise.

53.2.5 Properties of Plastics

Most of the properties discussed in the following sections are explained where first used. One key property relevant to packaging equipment, slip, is not covered in detail, so will be explained here. From the perspective of machining, plastic film must have the right friction coefficient to pass smoothly through the packaging equipment. If the slip is too high, the result is different package sizes in tube-formed packaging due to the film sliding past the forming rollers as well as a loss of registration in printing (see later). The product slides easily inside the package, interfering with sealing the pack. On the other hand, too low a slip can cause the film to stick to hot surfaces or folding box surfaces and can cause the product not to slide down to the bottom of the pack, also affecting pack sealing and presentation. The product should be able to slide back and forth in the pack relatively easily for tight sealing at both ends. This section also serves to introduce and describe the primary packaging plastics.

53.2.5.1 Polyolefins

53.2.5.1.1 Polyethylene

Polyethylene (PE) is the result of the polymerization of ethylene gas and has the formula $(CH_2)_n$. It was invented by ICI in the 1930s. Two main manufacturing processes result in different polyethylene products. The first is called low-density polyethylene (LDPE), and the second is high-density polyethylene (HDPE).

LDPE is formed at high pressures (1000–3000 atm). This results in long branched chains, weakly linked to each other by van der Waals forces (but strong overall force due to length). The branching is random, and so LDPE is an atactic polymer. Thus neighboring chains can slip past each other, allowing the material to bend easily (be flexible). As a result, the printability of LDPE is poor. However, many plastics with poor printability can be made printable by corona treatment, in which an ionic discharge is used to sensitize one side of the plastic.

LDPE has low density, as the long chains exhibit branching, so that the molecules are not able to fit closely together. This irregularity in structure also results in a lower melting point and a less crystalline (ordered chain) structure. LDPE is tough, semitransparent (poor clarity), flexible, and has a waxy feel. It resists most chemicals below 60°C, and it resists water moderately but not gases (poor O_2 barrier). LDPE is usually used as thin sheets or laminated to other packaging materials. It is used for bag manufacture (bread, diapers), for low-temperature storage (due to its low barrier properties), and for packaging rice. The melting point of LDPE is 105°C.

HDPE is produced at low temperatures and pressures of about 10 atm. This gives rise to an ordered molecular structure, which is called an isotactic polymer. The Ziegler process is used, employing a catalyst. HDPE is stiffer, harder, less flexible, and waxy. Higher temperatures are required to produce thermoplasticity (melting point = 134°C). HDPE is used for making containers, e.g., crates, bottles, bags, tubs, and plastic knives and forks. It can be steam-sterilized, whereas LDPE cannot. HDPE bottles are opaque and can be used to contain detergent and milk. HDPE resists fats and oils better than LDPE. However, it does not seal easily.

This description simplifies the total PE picture. Available PEs include low-, medium-, and high-density PE, linear low (LLDPE) and medium densities (with twice the hot-tack strength), copolymers of PEs, copolymers with vinyl acetate (EVA), and ionomer film (surlyn). PE may be coextruded with nylon, saran, and ethylene vinyl alcohol (EVOH) sandwiched inside. Such films have high strength, flexibility, clarity, and especially barrier properties, and these are used for bag-in-the-box, pouches, cups, and lids. The form of PE used will usually be a trade-off between barrier properties required and cost.

53.2.5.1.2 Polypropylene

The monomer polypropylene (PP) has the formula $CH_2CH–CH_3$. PP was developed using polymerization catalyst technology by Guilio Natta in 1954. PP forms a regular, highly ordered polymer at low pressures in the presence of certain catalysts called isotactic polypropylene. It has high crystallinity (high clarity and gloss), is hard, heat resistant (higher softening point, 150°C), exhibits good memory, flex crack-resistance, puncture-resistance, and stiffness. It is resistant to chemicals (except aromatic and chlorinated hydrocarbons).

PP has excellent moisture and relatively good gas barrier properties. It can be printed on and is ideal for reverse or surface printing. Cast polypropylene has excellent heat sealability. PP is used for injection-molded containers and blister packs, laminations, carton overwraps, snack food bags, and confectionery bags. It may be coated (e.g., with polyvinylidene chloride or acrylic) and may contain additives. PP has poor heat stability, so precise heat control is required in the packaging equipment.

PP film may be stretched during production yielding an oriented (OPP) or biaxially oriented film (BOPP) of high clarity, strength, and resistance to water vapor and gases (e.g., for wrapping snack foods), and coated with saran or acrylic for better barrier and heat sealability. PP causes contact transparency ("show-through") and is available in thicknesses from 15 to 75 μm. It may be pearlized ($CaCO_3$ + heat generates CO_2 bubbles, resulting in reduced barrier properties and strength,

but no show-through) or white (pigmented, better print density, saves undercoat, reduced show-through). It is used for confectionery, especially chocolate. PP may be coextruded with PE (1.5 μm) for heat sealability.

53.2.5.1.3 Polyvinyl Chloride

The monomer polyvinyl chloride (PVC) has the formula $CH_2CH–Cl$. The term vinyl means that a halogen has been substituted for a hydrogen atom. PVC has low crystallinity (so has good transparency when pure), but higher interchain bonding than PE due to the Cl^- halogen, so it is harder and stiffer. For this reason, plasticizers may be added during manufacture. The Australian Standard 3010—Plastics for Food Contact: PVC allows four plasticizers for food involving flexible sheets and films, e.g., vitafilm (for meat) with particular oxygen diffusion characteristics.

PVC has good feel and printability. It is highly inert. It is glossy and resistant to moisture, fats, and gases. There is a great variety in PVC compositions, e.g., stabilizers, impact aids, lubricants, and other additives are present in large proportions. The stabilizer is necessary because the decomposition point for PVC is close to the melting point. For food products, the extraction of the stabilizer from the PVC must be less than 1 ppm of the stabilizer, and the stabilizer must be a calcium–tin or dioctyl–tin system (not butyl–tin, used for nonfood products), containing tinuvin for UV protection. PVC is used in the biaxial-stressed form, e.g., for shrink-wrapping of cheese and meat. It is also used for thermoformed containers, e.g., for chocolates, as well as for plastic pipes and toys. It heat-shrinks after stretching and can be thermoformed.

53.2.5.1.4 Polyvinylidene Chloride

The polymer polyvinylidene chloride (PVDC) is similar to PVC, except that there is a double chlorine substitution, giving $CH_2=CCl_2$. PVDC has a more ordered structure, with high crystallinity and softness. It has excellent barrier properties (especially to O_2) and it is commonly used as a copolymer with PVC. PVDC possesses reasonable clarity, good feel, good printability, and strength. It is difficult to cut as it lacks stiffness, so it is hard to machine by itself and is too expensive for use as a pure monofilm (except for household use). It resists chemicals; has low water, gas, aroma, and flavor permeability (due to chlorine ions); and high strength. It has high chemical stability and is hygrophobic.

PVDC is used with PVC as a copolymer to coat other packaging materials to provide good barrier properties. It is used in laminates and is an important shrink film, with excellent cling properties. PVDC cannot be reprocessed because it degrades (melt point 162°C). This makes coextrusion lamination difficult as well, although it can still easily be used in coating from solution. When used in coextrusion it must be copolymerized first (for example with vinyl chloride) to give better temperature stability.

53.2.5.1.5 Polytetrafluoroethylene

The formula for the monomer polytetrafluoroethylene (PTFE) is $CF_2=CF_2$. Polymerization produces long, straight unbranched chains, with ionic intermolecular bonding, so PTFE is strong and crystalline. Since the bonds C–C and C–F are strong, this material is extremely inert and has a high softening point (340°C). PTFE possesses a high gloss and is waxy in feel. It is used in coating cookware (nonstick surfaces) where easy-to-clean nonstick surfaces are required.

53.2.5.1.6 Polystyrene

The polymer polystyrene (PS) results when an ethylene hydrogen is replaced by a phenyl radical ($CH_2CH–C_6H_5$). It is a synthetic rubber, which does not degrade over time. PS is amorphous (random packing), low in density, and brittle. In the pure form, it has good clarity and printability. It is often used for loose bulk packaging, especially for packing fragile materials. Originally, this involved aeration of the liquid plastic with fluorocarbons, but environmental aspects have encouraged CFC-free production and reusability of loose packaging materials (also called void packaging).

PS is usually copolymerized, especially with butadiene, to give high-impact polystyrene (HIPS, see later), which has a less brittle structure. Yogurt and ice cream tubs are common examples. Margarine tubs may be made from acrylonitrile buta-styrene (ABS). PS is also used for disposable plates and cups. PS can be foamed with hexane to form expanded polystyrene (EPS), a low-cost, low-density material that is easily formed into holding trays, cups, etc.

Polystyrene is the plastic of choice for thermoforming because it has strength, low cost, formability, and sealability. PE and PP are only used where specifically required; PP containers can be filled at high temperatures. Although PS provides a good barrier to gases, it is permeable to water vapor. It resists grease, acids, alcohols, and alkalis (i.e., chemically stable). Oriented polystyrene is a very useful packaging film and is heat shrinkable. The melting point of HIPS is 100°C.

53.2.5.2 Other Thermoplastics

53.2.5.2.1 Polyesters

Polyesters are plastics formed by the polymerization of esters. In general, they have reasonable clarity and poor feel and printability, but are strong, versatile, with good heat resistance. This is useful for boil-in-the-bag type applications, where the plastic must sustain temperatures of 100°C without deforming or softening. When metallized polyesters are also used for snack and coffee pouches.

Ethylene glycol and terephthalic acid yield polyethylene terephthalate (PET). PET has high strength and chemical stability. It is used for blow-molded bottles and some films, increasingly for thermoformed trays, for shrink-wraps, and for boil-in-the-bag products. It has excellent clarity, comparable to that of glass. It can be printed, metallized, and laminated. It has high barrier properties for a plastic film. Due to efficient recycling, PET is a cheap plastic. It is only used in 12 and 15 μm gauges for film and is commonly used as the outer layer of a laminate structure due to its gloss and temperature stability (melt point 254°C). It is used for microwave pie wraps (microperforated).

Forms of PET include APET (atactic PET), CPET (crystalline PET), OPET (oriented PET), and PETG (copolymer PET/

cyclohexane dimethanol; melting point 265°C). Polyethylene naphthalene (PEN) shows promise due to its low permeability to gases. There is growing interest in recycling polyesters due to their initial high cost. The advent of the PET range has revolutionized the packaging industry, allowing plastic to compete directly with glass bottles. Blow-molded PET bottles have less weight, lower production and energy costs, are droppable, and still have excellent clarity and gloss. The bottle is molded as follows: the closure is first injection molded, producing a little bottle with the screw top already formed, then transferred hot to a blow-mold die. The wall thickness is computer-controlled. The production equipment is specific to PET. Current production rates are about 1/sec. They are not suitable for beer, which requires a light barrier (opacity).

53.2.5.2.2 Cellulose

Cellulose was the first transparent film to be used (invented by Du Pont) in packaging and was widely used until the advent of polypropylene. It is biodegradable. A common name is cellophane, a trademark name of British Cellophane Ltd. Cellulose is clear, stiff, printable, and glossy, but has poor feel and moisture resistance. It has good heat-dimensional tolerance. It is not heat-sealable. It is naturally antistatic, so is good for powders (e.g., milk powder). Cellulose is still widely used today, due to the addition of coatings that have given cellophane great adaptability. Examples of coatings are nitrocellulose (making cellophane flexible and durable), PVC, PE, and PVDC (oxygen-barrier properties).

Cellulose can be laminated to foil, paper, and some plastic films. It is able to retain folds (called *dead fold*), so that if it is twisted, it retains that twist, making it ideal for individual candy wrappings. It is also tearable. It is used for cookies, confectionery, and pastries in situations where vapors may need to "breathe" to prevent surface molding. Cellulose is sold based on weight per unit area, not thickness, due to the variation in thickness that occurs with moisture absorption. It is available in weights of 22–60 g/m^2. Some typical cellulose codes are given in Table 53.4.

53.2.5.2.3 Cellulose Acetate

Cellulose acetate is made from cellulose and acetic anhydride. Cellulose acetate has good clarity and is printable in sheet form, but has poor feel and barrier properties. It is becoming obsolete, although it is still used in laminates and thermoformed blister packs.

53.2.5.2.4 Other Thermoplastics

Other biodegradable biopolymers are being developed but have not yet become competitive. The raw material for the new thermoplastics is usually starch, which is blended with conventional oil-based polymers.

53.2.5.2.5 Polyamides (Nylons)

Polyamides (nylons) are made from condensation of a diacid (e.g., adipic acid) and a diamine (e.g., hexamethylene diamine). Polyamides have high crystallinity, strength, impact strength, puncture and stress-crack resistance, flexibility, and melting (255°C) and softening points. They also have good chemical resistance. Polyamides are used for boil-in-the-bag–type products, frozen foods, fish, meat, vegetables, and processed meat and cheese, always in lamination. They have low water barrier and high gas permeability. A large range of different nylons exists. Polyvinylidene-coated or metallized nylon is about twice the price of pure nylon film. Nylon is available as cast (18–100 μm) and biaxially oriented (BON) nylon (12, 15, and 20 μm). It can be metallized or PVDC-coated for better barrier properties and is usually laminated. As a film, it must be biaxially oriented to give printing and machining stability.

TABLE 53.4
Some Typical Cellulose Codes

Code	Explanation
A	Anchored (describes lacquer coating)
/A	Copolymer coated from dispersion
B	Opaque
C	Colored
D	Coated one-side only
F	For twist wrapping
M	Moisture-proof
P	Plain (non-moisture-proof)
Q	Semi-moisture-proof
S	Heat sealable
/S	Copolymer coated
T	Transparent
U	For adhesive tape manufacture
X	Copolymer coated on one side
XX	Copolymer coated on both sides

53.2.5.2.6 Polycarbonates

Polycarbonates are formed from condensation of carbonic acid in the presence of aliphatic or aromatic dihydroxy compounds. They are amorphous. Most polycarbonates are tough, stiff, hard, and transparent (high clarity), with high softening points, and so can be cook in the oven or sterilized. They also have poor barrier properties and cost three times as much as polypropylene. They are used for plastic tableware and fruit juice containers.

53.2.5.2.7 Acrylonitrile

Acrylonitrile (AN) is an excellent gas barrier (like EVOH and PVDC). It possesses good chemical resistance and has a melting point of 170°C. It is easy to thermoform because of its high stiffness. Acrylonitrile forms copolymers such as Lopac and Borex, which have potential as high barrier plastics. Acrylonitrile buta-styrene (ABS) is still used, but polyacrylonitrile (PAN) has been replaced by PET.

53.2.5.2.8 Plioflim

Pliofilm is a rubber hydrochloride formed by combining polyisoprene (natural rubber) with hydrochloric acid. It is a printable, good-feel, opaque film with good heat-sealing

characteristics and grease resistance. Pliofilm is no longer used much, as it is not easy to machine and is not very durable. However, it was one of the first thermoplastics. It is chemically stable, requires a plasticizer, and has poor barrier properties.

53.2.5.2.9 Ethylene Vinyl Alcohol

Ethylene vinyl alcohol (EVOH) is a film that has high oxygen barrier properties, but hydroxyl groups make it hydrophilic, which increases its permeability. Thus, it must be sandwiched between materials with good water barrier properties, such as polypropylene of low-density polyethylene, to be effective. However, its oxygen barrier properties make it a highly desirable film, competing with PVDC for this role. EVOH is more expensive than PVDC, but it is easier to process and is recyclable. The melting point of EVOH is 185°C.

53.2.5.2.10 Ionomers

Surlyn is the brand name of a range of Du Pont ionomer resins (invented by Rees in 1961). An ionomer resin has both ionic and covalent bonds. The ionic bonds are due to sodium or zinc cations. Surlyn has low-temperature heat sealability (about 90°C), good hot tack (four times better than LDPE, LLDPE, and EVA), formability, toughness, and chemical inertness. Surlyn has some haze. To achieve a coefficient of friction of about 0.2 (as required by modern packaging machinery), slip agents must be added. Surlyn is used for shrink-wrapped meat, cheese blocks, fish, individual candy wrapping, pet food bags, potato chips, snack foods, drink Tetra Pak cartons, margarine tubs, cookies, frozen foods, and nuts as part of a laminate structure, especially as the inner heat-sealing layer.

53.2.6 Processes of Plastics

53.2.6.1 Copolymers

Condensation of combinations of certain homopolymers with each other can produce complex copolymers with properties different from the individual constituents. Depending on how the individual monomers combine, a great variety of properties can result. Let A and B be two monomers: They can combine (i) in strict sequence, e.g., A–B–A–B–A–…; (ii) in blocks, e.g., A–A–A–A–B–B–B–B…; or (iii) in branching chains, with a central chain of one monomer. Some examples are ethylene-vinyl acetate (EVA, a good heat sealant but with poor barrier properties), ethyl–vinyl alcohol (EVOH, a hygrophilic plastic), vinyl chloride copolymers, and polystyrene copolymers.

53.2.6.2 Lamination

Since no single film can satisfy all packaging requirements, plastic films may be combined by lamination or coextrusion. Lamination is a technique for bonding films to produce a film with the properties of both constituents. By combining the qualities of choice from the raw material films, a laminate can be tailor-made for its particular application. Each layer in the resulting laminate may exhibit different properties from its free state, such as mutual layer reinforcement in which cracks in a brittle layer are prevented from propagating by a high elongation (elastic) layer. This effect depends on good adhesion between the layers. Three factors that affect the adhesion between layers are given in Table 53.5.

TABLE 53.5
Factors Affecting the Adhesion between Layers

1. Viscosity/shear rate match during melding. To be coextruded the melt flow viscosities should be similar (a ratio of within 3:1), otherwise one of the plastics will flow with respect to the other, preventing bonding.
2. Temperature, pressure, and period of contact, to build the bond.
3. Functionality of adjacent resin layers, i.e., that they are sufficiently similar in structure to mix at the contact surfaces.

If these factors are not all present, then an adhesive layer is necessary and the plastics may be cold-bonded with a tie-layer of resin adhesive. Adhesives are discussed later. A typical triple-layer film would be composed as follows:

- Properties of outside layer—high gloss, printable, good lamination, possibly metallized, high slip
- Properties of middle layer—strength, stiffness, barrier properties, possibly opaque
- Properties of inner layer—easy to seal (hot seal, good hot tack properties, or good cold seal properties), low migration rates, barrier properties

A laminating machine has the following components:

1. Continuous feed roll with a feeder "on-the-fly" splicer, which can cut off the old roll and join on the new (there may be several rolls feeding film into the machine at once.)
2. Tensioning rollers to give exact control over the tension in the plastic.
3. Lamination stage where the primary and secondary web are combined (web refers to the film as it passes through the machinery).
4. Compression rollers to push the layers together.
5. A take up (rewind) roller to collect the final laminate.

Note that plastics can be laminated with papers and foils as well as plastics. Laminations with paper will tend to use water-based adhesives, since the solvent (water) will absorb into the paper base away from the adhesion zone, allowing the glue to set quickly. Lamination also allows reverse printing, where one plastic layer is printed (in mirror image) before lamination in order to sandwich the printing inside the laminate for greater protection. Lamination of plastic films is achieved by one of four processes:

- Adhesive lamination, where a continuous glue source is fed as a flat film between consecutive layers of the laminate. This may be a hot glue (a plastic resin fed from an extruder, for example) or a pressure-sensitive

glue applied as a high-viscosity liquid to one web, then dried in a tunnel oven to remove the solvent, and then wrapped with the secondary web around a chill roller. The glue may physically bond (e.g., to paper or board) or chemically bond. This technique is also used for labels.
- Extrusion lamination, where extruders replace the feed roll, and the layers are coextruded.
- Thermal lamination, where heat is used to join the webs by partially melting.
- Wax (or hot melt) lamination, where a thermoplastic glue or wax is used instead of a pressure-sensitive glue. The rollers must have the capacity to be heated to a suitable temperature.

Typical speeds for lamination are 100–200 m/min.

53.2.6.3 Coatings

To enhance plastic film properties such as printability, coatings are often used. Aqueous and solvent coatings are applied to the substrate through water dispersions or emulsions, solvent solutions, or waxing. Aqueous and solvent coatings are applied to the substrate using direct roll, direct gravure, or reverse gravure methods. The coating liquid is then dried and cured by infrared heat or a hot-air oven. The coated film is then cooled before being wound onto a roll core. Modern machines allow simultaneous coating on both sides. Examples of coating are foil lidding, reverse gravure, cohesive coating, lacquer coating, and paraffin coating.

Foil lidding is a lacquer coating applied to the foil, which can be heat-sealed to the container but is peelable for convenient opening. On the outer side, a clear nitrocellulose lacquer is applied. PVDC may be coated onto paper or PP by reverse gravure. PVDC does not bond well to other plastics, so a prime coat may be applied first. The prime coat may be nitrocellulose, vinyl, acrylic, or shellac. Cohesive (cold seal, latex-based) coatings are often gravure-coated onto films during printing (on the opposite face). Cold seals are pressure-sensitive, so they can be sealed more quickly than hot seals, which require adequate dwell-time. Colored vinyl lacquers may be applied to outer packaging surfaces, e.g., to give colored aluminum foil. Paraffin waxes are used as coating bases for papers, especially for inner wraps or liners for biscuits or cereals. The wax may be blended with resins, synthetic rubbers, and polymers to meet specific requirements.

53.2.6.4 Adhesives

Adhesive layers are used to bond film layers together to construct a laminate and are polymers. These tie layers will affect the mechanical properties of the final construction. They are usually expensive and must have lower melt temperatures than the layers they are bonding. The adhesives are mostly olefin copolymers, polyurethane, or polyester dissolved in solvents. If no solvent is used, the glue may be extruded hot between the films. Examples of hot-melt adhesives are copolymers of ethylene and acetic acid and surlyn.

Adhesives must "wet" the film surfaces to provide laminate strength, or the laminate will tend to separate into its individual components when used. They may be categorized as liquid, solid, solution, or emulsion [6]. Liquids are monomers that react with trace water to form polymers, such as cyanoacrylate ("superglue"), reactive liquids that combine chemically such as epoxy resins, and pressure-sensitive adhesives (congealed liquid resin in a rubber matrix, with the property that they can be peeled off again). Solids are powder adhesives activated by the addition of moisture, solvent, or heat. Hot melts consist of a polymer, resin, wax, and stabilizers. The hot melt is supplied in solid form, applied in the hot liquid form, and then resolidified as it cools. No solvent is required and they set very quickly. The two main classes of solutions are a water-based (traditional glues with natural polymers such as starch, flour, casein, animal glue, dextrin, gum arabic; and newer synthetic resins in solution such as polyvinyl acetate (PVA), cellulose ethers, and vinyl pyrrolidones), and other solvents (natural, e.g., resin, shellac, bitumen, rubber, and gums, and synthetic, e.g., nitrocellulose, urethanes, nitrile rubbers, epoxies, and cellulose acetate). Emulsions dry faster than solutions and have a greater range of properties. Synthetic resin emulsions now dominate the packaging industry. They include PVA, acrylic resins, and polychloroprenes.

53.2.6.5 Heat Seals

A major requirement of plastic film is heat sealability. A heat seal is the fusion of two surfaces under the influence of heat, pressure, and time. The two surfaces are partially melted by the heat applied by a pair of heated jaws. For plastics such as polypropylene, which has a higher melting point, a copolymer coating may be applied on one or both sides to give heat sealability. Some important terms used in heat sealing are as follows. Dwell time for heat is to penetrate outer films to reach the two layers being bonded, i.e., time the plastic must remain under heat and pressure. Heat seal threshold is the minimum temperature at which a heat seal threshold of 200 g force per 25 mm is obtained. This temperature should be about 80–120° C, i.e., a balance between too high (wrong layers melt) and too near ambient (no melting). The lower the temperature, the faster the sealing, since less heat must be lost before resolidification of the plastic. The pressure applied is about 1 atmosphere. The useful heat seal range is the temperatures over which the films may be sealed. The upper limit is the point where distortion of the plastics starts to occur. Maximum linear film speed is the maximum speed at which film can be passed through the sealing machine. Hot tack is the strength of the initial partially molten seal. If the hot tack is not good, then stresses on the film before cold tack is achieved can reduce the integrity of the seal. Cold tack is the final "cold" strength of the bond.

53.2.7 Plastic Package Types

53.2.7.1 Plastic Bags

Bags are formed from sheet or film plastic by folding and heat-sealing as required. Some bags have folds in the base so that when packed, they expand to a rectangular shape. Handles may be inserted during folding and heat-sealed into the folds.

The bags must be strong enough to resist breakage under the design load, but also must not break when being loaded.

Bags may be preformed, in which case they may be "wicketed," or formed from the source plastic sheet during packaging (usually by forming tubes from the plastic). Wicketing is the process of punching small carry holds at one end of the bag with which to hold the bag during loading. The holes must be carefully designed to carry the load of the product entering the bag, yet must tear off easily so that the next bag becomes available.

53.2.7.2 Plastic Closures

A closure must perform five functions:

1. Contain, to the same level as the remainder of the package.
2. Allow access, so that the consumer can retrieve the product in a convenient way. The ability of the package to be functional in this regard is an important marketing consideration.
3. Restrict access, e.g., tamper-evident and child-resistant caps.
4. Protect the product, such as keeping out dirt and moisture.,
5. Be economic.

The closure may also be used for advertising or barcoding. A plastic screw cap lid has three main components: (i) the cap itself; (ii) a linear (HDPE wad), adhesively attached to the cap in most cases; and (iii) a screw, which interlocks with connecting lugs in the finish of the container but does not provide a good barrier seal.

The closure must be applied with the correct torque. Insufficient torque leads to leakage, whereas too much torque makes removal by the consumer difficult. Tamper-evident attachments to the screw cap are commonly used with plastic beverage bottles, consisting of a ratchet ring under the cap, which becomes detached when the customer removes the lid. A dispensing closure is one that allows the product to be dispensed without removing the closure. Examples of dispensing closures are lids such as flip-tops (e.g., gable-top cartons), pump action, aerosols, and opening pourers, which allow small amounts of the product to be easily removed.

Aerosols are cans containing a liquid product layer and compressed gas propellant. The propellant may also be present as a liquid, which boils as product is used to replenish the driving pressure. The product may be dispensed as a fine spray, mist, dust, or foam. An example of an aerosol application for the food industry is in instant whipped cream. The advantages of aerosols are simple dispensing and complete exclusion of air. They are, however, expensive and an explosion risk, and since 1974 the use of CFCs has been a suspected cause of damage to the ozone layer. There is now an almost total ban on CFC propellants. In Australia, CFCs have been totally replaced by trigger pump packs and inert gas pressure packs.

The advent of flexible packages (replacing rigid containers) has led to a need to develop closures for such packages. Methods include plastic zippers, pressure-sensitive tape, metal bands, plastic clips, and twist ties (e.g., breads). Child-resistant lids have locking devices that either prevent turning unless squeezed or require pressure to open. Tamper-evident seals are discussed in more detail elsewhere.

53.2.7.3 Oven-Safe Containers

Plastic-based food trays that can be heated have become an essential component of convenience foods. The three main plastics used are polypropylene (PP), polystyrene (PS), and crystallized PET (CPET). PP is suitable for microwaves but not for the higher conventional oven temperatures. Foam PS has a still lower melt temperature, but now PS low-density blends have been developed with heat deflection temperatures (HDTs) of 190°C, which is suitable for microwaves. These are cheaper than CPET. CPET is stable from –40°C to 220°C, is clear and resists fats, oils, water, and oxygen on the shelf, but is more expensive. Some instant snack meals have complex laminate structures with metal FAEZO (full aperture easy opening) lids and snap-on plastic cover lids. Aluminum foil trays have good appearance, stack well, have good barrier properties, and can be dual ovenable. Paperboard trays are ovenable in a range of sizes.

53.3 METALS (STEEL, TIN, ALUMINUM)

Steel, tin, and aluminum are used mainly for canned foods and beverages. The most common use of metals for packaging is in tin-coated steel and aluminum cans. The principal advantages of metal cans are their strength providing mechanical protection, effective barrier properties, and resistance to high temperatures providing stability during processing. As regards their opacity, it is an advantage for light-sensitive products, but a disadvantage in that the contents are invisible. Other disadvantages of metal cans are their heavy mass, high cost, and tendency to interact with contents and environment (internal and external corrosion). The critical concepts of canning are to ensure that the product in the can is stable and that the seal provided by the metal is complete. Tin coating or lacquering is an important part of can manufacture. The lacquer is a resin, such as an acrylic (which resists high temperatures), oleoresinous, alkyd, epoxy, phenolic, polybutadiene, or vinyl resin. More than 200 different protective coatings are now in use [7]. The lacquering must be complete. Small gaps in the coating can lead to the iron being exposed. The interior coating has to withstand sterilization temperatures and action of acids, as well as sulfide staining. As iron corrodes, it produces hydrogen gas, which can blow the can. The development of lacquers has meant that tin-free cans are possible. Another disadvantage of steel is the high-energy requirement during manufacture [2, 3].

Aluminum is used increasingly for canning, due to its lightness, low cost, corrosion resistance, availability, and recyclability. Aluminum is also used extensively in many non-canning applications, such as foil packaging, caps, convenience food containers and lids, yogurt tub lids, kitchenware, and laminates. Foil may be used for formed or semi-rigid

containers. Aluminum foil is difficult to use on modern fast-packaging equipment because of creases, tearing, and marking effects. In practice, aluminum foil of fine gauge may have minute pinhole defects due to the tolerances of the rollers, crystal size, and lubricants used, which allow transmission of air and water.

53.3.1 Canning

The most common use of metals for packaging is in tin and aluminum cans. The metal provides a highly effective barrier between the food product and the environment. Thus, the critical concepts of canning are to ensure that the product in the can is biologically stable and that the seal provided by the metal is complete. Food stability for nonpowders is usually achieved by thermal processing. Canning was invented by Appert in the nineteenth century in response to the need to supply Napoleon's army with good-quality food. He used glass bottles, but Durand (English) used metal and pottery at about the same time. The two ideas together gave us tin cans.

Tin cans are made of sheet steel coated with 0.5 mm tin. The coating is applied by tinning, which is electrolytic deposition of the tin at about 10 g/m^2, and hot-dip, which uses about 30 g/m^2. The steel is rolled and ribbed (for added strength) and either sealed with solder (usually 95% lead, 5% tin), or, more commonly, welded. The resulting tube ends are flanged, and the lids at both ends are attached by a double seam without solder. Since steel corrodes rapidly in the presence of acidic substances, the tin acts as a barrier. Some cans are lacquered internally for high-acid products (pH <3) or for products that change color in the presence of tin. Foods that contain sulfur produce a blackening of the tin. The steel can provide almost perfect barrier protection and, due to its structural strength and ability to handle pressure, can be retorted (cooked under pressure) after sealing.

In competition with traditional tin-plated steel cans, modem cans may be made from tin-free steel, aluminum, or laminates. Laminates or composite cans are often fiber–foil containers, such as helical-wound tubes, with metal ends. The fiber may be paperboard. The first layer would be the liner, followed by other layers until finally the printing layer is wound on. The layers are sealed together with adhesives, which generally contribute the majority of the structural strength.

The closure is traditionally the seamed lid, to be opened by the consumer with a can opener. A major development in canning has been the customers' preference for convenience over cost, so that pull tabs (now out of favor because of pollution), zip tops, pop tops, and ring pulls (where the ring remains attached to the can) have been adopted. Other examples are sardine cans (peel-back lids using a key to lever open the lid) and the recently developed full aperture easy opening (FAEZO) cans, where a ring tab is used to peel back the entire top lid. The lid is scored during manufacture to a precise metal thickness; if too thick, the can is difficult to open, and if too thin, the package integrity is endangered.

Not all cans are retorted. The term "general line" is used for containers that are not hermetically sealed for heat processing, which accounts for about 16% of the tinplate market worldwide. The advantages of tinplate are strength (impact, puncture), barrier, formability, printability, and product compatibility. They tend to be used for higher-value products, as the painted tin can look very effective. The processes of manufacturing steel cans are described well in many textbooks and will not be covered here. Aluminum can manufacture is described next.

53.3.2 Aluminum

Aluminum is used increasingly for canning, due to its lightness, low cost, corrosion resistance, availability, and recyclability. Aluminum is also used extensively in many noncanning applications, such as (i) foil packaging, e.g., chocolate, household or industrial foil; (ii) bottle closures and overwraps, e.g., caps, wine bottles; (iii) convenience food containers and lids, e.g., frozen-stored/oven-heated, single portion sizes, yogurt tub lids; (iv) kitchenware, e.g., saucepans, cutlery; (v) special applications, e.g., shrimp freeze blocks; and (vi) laminates.

53.3.2.1 Properties

Aluminum makes up 7.9% of the earth's crust and is attacked by acidic solutions (especially food acids: pH < 4). Special inks had to be developed to work with aluminum, due to its smooth metal surface and high reflectance. A common solution with formed containers is to put all of the print on the lid. The main properties of aluminum are lightness (three times lighter than steel), strength (alloys are as strong as steel), corrosion resistance, electrical conductivity (twice that of copper), appearance, and ease of recycling. It has the barrier properties of steel but without the corrosion problem. It is highly attractive in appearance, as it reflects about 85% of the incident light, and so stands out from other products. It can be bonded with paper (e.g., chewing gum and cigarette wrappers) to allow easier printing. It has excellent strength, so that thin films can be made. It can be extruded into complex shapes, such as roof guttering.

53.3.2.2 Manufacture

Aluminum foil became common after the electrolytic method of extracting aluminum metal from bauxite was developed independently by Hall in the United States and Heroult in France in 1883. Bauxite contains 75% hydrated alumina (Al_20_3) in mono- and trihydrate forms, as well as oxides of iron, silicon, clay, etc.

53.3.2.3 Aluminum Foil

Foil may be used for formed or semirigid containers. Many instant meals are packed in cooking and eating trays made of aluminum, with different compartments commonly formed in the tray to separate the meal components, especially with frozen foods. Aluminum foil is made of solid sheet aluminum rolled to a thickness of less than 0.15 mm and sold on cardboard rolls. For food applications, it is sold at high purity (99.8%) except for formed containers, where it is strengthened (like steel) by the addition of 1–1.5% manganese. After

rolling, the aluminum is work-hardened, so it is brittle and crinkly. The aluminum can be softened by slow heating and slow cooling (annealing, about 24-hour heating at 300°C, which also cooks off the processing lubricating oils, then 24-hour cooling is typical), giving a soft metal of low strength and high flexibility (ductility) suitable for household use and most other food applications—this is called zero temper. By rolling and strain hardening ("quenching" when hot), a more brittle, stronger material is produced. This is called H temper, with a number added according to the degree of hardening. Hard tempers must be used whenever a high degree of forming is required. The metal may also be normalized, a process of air-cooling heated metal that is intermediate between quenching and annealing.

53.3.2.4 Foil Laminates

Aluminum foil is difficult to use on modern fast packaging equipment because of creases, tearing, and marking effects. Thus, additional treatments are common. Lamination can be difficult for the same reasons, but once laminated the resulting plies have excellent machining and visual properties. For example, sodium silicate may be used to glue foil to vegetable parchment (cigarette foil). The foil may be printed, coated, seal applied, and laminated in a step called converting. The web may also be embossed (embossing roller), giving a textured matte appearance, which reduces glare and makes separation in refrigerated storage easier.

53.3.2.5 Rigid Foil Containers

Rigid aluminum foil containers have varying strengths but offer the ultimate in convenience for all food processing, packaging, display, and consumer requirements. They are produced as follows. The foilstock (after hot-rolling) is cold-rolled to 7 μm, the final rolling being two-ply. The plies are separated and annealed (i.e., cold-rolling causes high degrees of work hardening). The foil may then be coated. The web is then slitted to size and then die-formed using complex dies, which control the degree of drawing of the aluminum to retain uniform strength. Lids may be cardboard laminated with aluminum. They are not affected by heat and can be heated, immersed, and frozen.

53.3.2.6 Pinhole Defects

In practice, aluminum foil of fine gauge may have minute pinhole defects. The aluminum foil is graded according to the number of defects as: grade 0, <200 holes/m^2; grade 1, 200–900 holes/m^2; grade 2, 900–3000 holes/m^2; and grade 3, >5000 holes/m^2. The size of the holes is also significant. For this reason, aluminum is commonly bonded with polyethylene for commercial food-packaging applications so that thicknesses of the order of 10 μm can be used. The resulting barrier properties are far superior to those of plastics and plastic laminates,

53.3.2.7 Aluminum Tubing

Aluminum is also used for squeezable tubes (e.g., toothpaste or tomato paste tubes). However, this use is becoming less common due to the following problems: (i) neck finishing is expensive; (ii) inks tend to crack and peel off after squeezing; (iii) some products are affected by aluminum, so a lining may be necessary; (iv) plastic laminates cost about 20% less; and (v) aluminum tubes are more subject to contamination. Plastic laminates (usually including a foil layer) are increasingly being substituted for products such as sauces, peanut butter, and cheese. The main problem with aluminum tubing has been the integrity of the side seam.

53.3.2.8 Aluminum Cans

Cans are made by cutting a blank (a disk of aluminum) from a coiled sheet, the skeletal web being recycled, and then drawing the blank into a cup. The walls may then be ironed by forcing the cup through a series of annular rings (dies) until the cup has the required height (drawn and wall-ironed [DWI] process), with a bottoming die forming a raised dome in the base. Alternatively, the cup may be redrawn, giving a thicker wall (drawn and redrawn [DRD] process). The thinner-walled (DWI) cans are suitable for carbonated beverages, while DRD cans are suitable for steam sterilization and retorting. The body shell is trimmed to length, chemically washed, and then given a chemical etch primer (chromate phosphate) so coatings stick when applied. The lid (two-piece design) is then added.

The can may have a reduced neck diameter for improved appearance, better stacking, and saving metal. Ring-pull tabs or FAEZO openings may be used. Printing may be done before drawing the can (i.e., on the blank), the ink design stretching with the can, or may be applied by offset printing to the final can. Lacquers (e.g., vinyl or epoxy) may be applied internally for acid products to prevent interaction between the product and the can, and externally for protecting the ink and providing the right slip properties on the base. The final can has a base thickness of about 0.020 inch and a wall thickness of 0.0065 inch.

Some areas of current research are dent recovery (using laminates), foil machinability, pinhole defects, closure opening force, and recycling (Australia has the highest recovery rate in the world). Apart from cans, aluminum recycling is difficult due to lamination with other materials, food and moisture contaminants, and the low value and volumes of material, so the trend is to reduce the amount of aluminum required. Foil food containers are difficult to microwave due to arcing and heat energy reflectance. However, there is some interest in developing aluminum food trays for microwave use. All packaging materials either transmit (glass, plastic, paperboard), absorb (susceptors such as metallized polyester laminated to paper, useful for browning and crisping), or reflect (metals). Thus, the tray must be open to the microwave energy at the top, so that the food cooks more slowly but more evenly.

53.4 GLASS

Glass containers used to be and still are considered as a prestigious means of packaging, and serve for the most expensive wines, liqueurs, perfumes, and cosmetics. It is highly

inert, impermeable to gases and vapors, and amenable to the most diverse shaping. In its normal state, it has the advantage of transparency, but where required it can be given different desired colors. It has complete as well as selective light protection properties. Its main disadvantages are its fragility, heavy mass, and high energy requirement during manufacturing. In addition to its marketing strength, glass has other advantages that give it muscle in today's marketplace. It is an excellent oxygen barrier and completely neutral in contact with foods. Glass also fits well into the modern recycling society, since it can be recycled indefinitely. Glass packaging technology has developed to the extent that strength, minimal mass, color, and shape all have been improved. While glass would not supplant metal and plastic in volume, it is finding an increasingly strong niche at the high end of the food spectrum [2].

53.4.1 History

Glass was first manufactured by humans thousands of years ago, possibly as an offshoot of pottery as glazes, and dates to 12000 B.C. Pressing glass in molds to form cups or bowls dates from 1200 B.C. and blow piping was invented by the Phoenicians in 300 B.C. In the third century A.D. clear glass was discovered, for example, cast glass using flat stones, used for church windows. Until the industrial revolution, glass was mainly used for high-quality tableware.

With mass production, however, glass started to become ubiquitous, first through the cork-sealed, narrow-necked bottle, then from about 1850 on the wide-necked jar and from 1920 the screw-top jar. The glass bottle is an almost ideal form of packaging for a large variety of products. It is inert to most substances; the product is visible; the cylindrical shape is good for loading, stacking, and holding; and it is cheap to manufacture and versatile in design.

Glass bottles are used for milk, jams, soft drinks, wines, beer, and spirits, and for many food products. Glass is highly inert, shows the product well, is available, easy to mold, cheap, has almost perfect barrier properties (including barriers to odors), and is recyclable. However, it is brittle, and some product loss will occur through breakage. Because it is fragile, high weights are required per product unit, and for a while, research was directed at reducing the high weight ratios by coatings (e.g., surlyn), which allow the glass to be handled at much higher packaging speeds. The coating reduced breakage, but this research ended when the PET bottle became available. More recently, environmental considerations have revived the idea. Over 75 billion glass containers are used annually by the food industries.

53.4.2 Glass Manufacture

Glass is the result of heating silica, soda ash, and limestone to over 1500°C, with the small addition of minerals for color or strength. As the mixture melts, the compounds fuse and become easy to shape. This may be done by sucking the melt into heat-resistant molds or by blowing semimolten glass into rough shape in a mold and then pressing this into a second mold where a jet of compressed air forces the glass into the final shape. Crystallization is prevented by quickly cooling the final product, so that the final product is amorphous and thus transparent. Annealing is a process of reheating the glass, and then gradually cooling to remove stresses (also used for metals). Safety glass is laminated and toughened.

Special glasses include Pyrex, produced by the addition of borosilicate and having resistance to thermal shock; amber glass, used to inhibit ultraviolet radiation for beer bottles; and crystal with added lead. (Note that wine can leach lead from glass!) Glass may be corroded by the application of hot concentrated alkali. Leaching tests for lead, cadmium, arsenic, and zinc are conducted on glass with high contents of these minerals; but in most cases, the fusion process of glass production prevents traceable amounts of these elements from escaping. In general, glasses are not retortable due to thermal shock and the expense of Pyrex glasses, but if retorted the glass must be cooled under pressure to prevent thermal shock.

53.4.3 Glass Containers

The main components of the container are the cylindrical main part, the bottom, the neck (called the finish), the closure (the screw cap), and the label. The cylinder shape is chosen for maximizing strength for a given volume (the sphere is a better shape, but not convenient for packaging). Glass is not well suited for sharp corners, as stresses tend to concentrate in areas of sharp curvature. The main components of the cap are a lacquer, wad, liner, and cover. Caps may be plastic or metal, and the type of closure might be thread, lug, friction, snap-cap, roll-on, cork, crown, twist-off, etc. The various types of glass containers have a range of names:

1. Bottles (most used)—round, narrow neck to facilitate pouring and closure, for liquids and powders.
2. Jars (wide-mouthed bottles)—neckless, allowing fingers or utensils to be easily inserted. Used for liquids, solids, nonpourable liquids such as sauces, jellies, and pastes.
3. Tumblers (open-ended jars)—shaped like drinking glasses. Used for jams, condiments, and jellies.
4. Jugs (bottles with carrying handles)—short, narrow necks designed for pouring.
5. Carboys (shipping containers)—shaped like short-necked bottles, usually used with a wooden crate holder.
6. Vials and ampoules (small glass containers)—occasionally used for spices, but mainly used by the pharmaceutical industry.

The main uses of glass for packaging are in milk bottles, condiments, baby foods, instant coffee, and drinks. Glass is not used for frozen products or for ground or roasted coffee because of breakage costs and the difficulty of vacuum flushing.

53.5 TIMBER, CARDBOARD, AND PAPERS

Pulp products are widely used in food packaging in the form of different kinds of paper, paperboard, laminates, and corrugated board. The main advantages of paper are its low cost, low mass, relatively high stiffness, and excellent printability; the main disadvantage is its high sensitivity to moisture, reflected in close dependence on the relative humidity of the environment [2].

The basic raw material for papermaking is cellulose. The cellulose molecule consists of a long, straight chain of glucose units. Paper can be classified according to its surface properties into machine-dried, machine-glazed, or supercalendered papers. Great importance must be given to coated and laminated papers, particularly for difficult packaging conditions. All demands regarding special performance as a barrier against water or water vapor, for example, can be met by combining paper and other materials. These are wax-coated, bitumen-coated, and plastic-coated papers. Other special types of paper include glassine, greaseproof paper, vegetable parchment, and waxed papers. Glassine is used extensively because of its inherent resistance to grease, oils, and fats and is the densest paper made [2, 3]. Paper laminations commonly used include paper/aluminum, paper/plastic, and paper/plastic/aluminum.

The use of wood in packaging today is rather limited, confined primarily to crates, large boxes, and pallets. Its major advantage is its strength, but it is quite expensive and cheaper alternatives such as corrugated board have been found adequate for many applications. Even pallets, which used to be made exclusively from wood, are made today in part from foamed plastics [2].

53.5.1 Timber

Wood is commonly used in box construction, but the use of wood for individual packaging (such as cigars) has decreased since the advent of plastics. Examples of timber for packaging are cases, boxes, and casks for long-distance transport.

53.5.2 Cardboard

The next choice of packaging material to be considered is cardboard. This may be a protective package (see previous section) or a presentation package. Folding carton construction consists of taking a two-dimensional flat piece of board (excellent for storage) and cutting, scoring, folding, and then gluing (or locking) it into a three-dimensional rigid box. The cardboard will usually be laminated to paper to allow printing and presentation.

For transport purposes, the fiberboard must resist relative humidity and temperature effects. Relative humidity affects the moisture content of the board (which is hygroscopic), raising it from 6–7% safe moisture (at manufacture) to 14%, at which point the board becomes like a piece of rag. Temperature strongly influences the rates of diffusion of gases and moisture into the package. Thus, the transport requirements will depend on ambient conditions. The board should be designed for the optimum lifetime of the projected job.

The various elements of cuts, tucks, locks, flaps, and folds may be assembled in an endless variety of ways, although the most common is rectangular for packing and storage convenience. Since cardboard is obviously highly versatile, the details for construction vary widely. The actual specifications may include (i) grade of board, ink type, and glue type (e.g., hot-melt glue or water-based); (ii) performance criteria, such as handling strength and crushing strength; and (iii) size.

One of the major uses of cardboard is as corrugated cardboard, a concept developed by Albert Jones in 1871, adopting the method for making ruffles from collars and paper for sweatbands in tall hats. Jones hand-cranked paper, and in 1874 Oliver Long patented gluing paper to both sides of fluted paper. Corrugated cardboard consists of two linerboards covering a central corrugated sheet. The linerboards are made of kraft, test liner, or low-grade pulp covered with kraft. The corrugated paper is made of straw paper or kraft paper. The main factor determining the board properties is the corrugations. A useful modification is the double- and triple-wall container; the corrugations on successive layers are usually parallel. The board is usually printed at the time of manufacture.

Choosing a carton for a specific job depends on the capacity of the carton to meet the requirements for that job. There is a trend to replace subjective tests (e.g., cartons must run on certain packaging machines) with more scientific objective tests (e.g., compression strength). The choice of carton for a specific job will depend on

1. Carton load, both the internal weight of the product and the external load applied.
2. Warehousing conditions, such as stack heights, ambient warehouse conditions.
3. Storage life.
4. Type of handling, e.g., fork, manual, palletizer.
5. In-use conditions (especially RH).
6. Item size, determining critical dimensions of the carton.
7. Maximizing pallet efficiency by using the available space.
8. Size and style—accuracy of the dimensions, which affects packaging machine performance (e.g., 1/64 inch), prefolds, print areas, etc.
9. Protective properties—different cartons protect against different agents, e.g., moisture or odor. These vapors can enter/leave through the cardboard itself, through the creases, through the glue seals or through the gaps between folds. Moisture vapor protection (MVP) is generally achieved through waxing the cardboard and is measured in terms of the resulting water vapor transfer rate (WVTR) measured in $g/m^2/day$.

A waxed board is difficult to print on, so the board may be laminated with white paper. The glue must be chosen that will seal within the time the box is in the packaging machine.

A common glue is dextrin, which is water-based, the water being absorbed into the cardboard as the glue sets. Hot glues may also be used. The inks used must be of the specified hue (and reproducible), resist fading, and resist rubbing.

53.5.2.1 Opening Cartons

For ease of use, some quick reliable method of opening the carton is usually built-in. Examples are perforated thumbnail openings, fold-and-tear openings designed to assist pouring, or designated areas for cutting.

53.5.2.2 Board Strength

The board must be strong enough for packaging, handling, storage, and intended use. It creates a bad impression with the user if the pack bulges, so bulge strength is important. The board must also resist compression and a degree of impact. The strength of the cardboard chosen for the protective package is related to the strength of the product package so that if a weak carton is used, a strong external box is necessary for delivery to the point of sale. Note that the air moisture content (relative humidity) has a large effect on the strength of cardboard. Other problems with cartons include pallet integrity, ropes denting boxes, weak cartons (bottoms fall out), pallet stack collapse (compression), overweight cartons, and forklift damage.

53.5.3 Papers

Paper bags were used in the 17th century. A bag-making machine was developed in 1852 by Wolle in the United States. The gusseted bag (1873) and multiwalled bag (1925) were later important developments. Paper is defined as sheets of material thinner than 0.23 mm and lighter than 220 g/m^2. Paper and board are produced from wood pulp (treated with calcium bisulfite or caustic soda to break down the lignin structure), rags, and other waste. Paper is decomposed by bacterial action over a period. Thus, paper is ultimately environmentally friendly. However, paper has had tough competition as a packaging material over recent years due to the extensive use of plastics. Treatments of paper to make it more competitive include paraffin and waxes (waterproofing) and plastic coating (added strength, and water and gas resistance). Paper can also be laminated with aluminum.

Paper is produced from wood pulp, treated by the addition of soda, calcium sulfite, or calcium sulfate (depending on the end-use). The pulp is milled into a continuous sheet and bleached with chlorine, caustic soda, and sodium hypochlorite. After drying, it may be treated with various chemical coats to enhance its performance. Paper is designated by the weight of a ream of paper of a given size. Usually a ream is 500 pages, but variation in size makes it difficult to directly compare two papers. For printing, the standard size is 24 × 36 inches (6 ft^2 or 0.55742 m^2). Some of the standard papers used are

1. Bond papers (17" × 22"): soda pulp, uncoated bleached, finished to give wet strength and a good printing surface.
2. Tissues (24" × 36"): lightweight semibleached or bleached, finished to give wet strength, with open or closed fiber formation.
3. Litho papers (25"× 38"): smooth printing surface but not as strong as bond paper, used in magazines.
4. Kraft papers (24" × 36"): unbleached equivalent to bond paper, but of heavier basis weight (and hence greater strength), and cheaper.

Other special types of paper include glassine, greaseproof paper (glassine paper that has not been calendered and is free of wood pulp, water-resistant, and heavily milled), vegetable parchment (boil proof and fat impervious, due to treatment with sulfuric acid), and waxed papers. This paper is not moisture-proof, so may be waxed or laminated.

Glassine is used extensively because of its inherent resistance to grease, oils, and fats and is the densest paper made. It is made from straw, which is pulped and purified, then hydrated at high temperature until it partly gelatinizes. The resulting sheet is fed into a calendar, where it is rolled under high-pressure steam to give a transparent paper. It can be laminated. It is used for dry products such as cereals and biscuits after being waxed (paraffin).

Paper laminations commonly used include paper/aluminum (for strength and excellent resistance to moisture and air) and paper/plastic (good for heat sealing as the plastic can bond across the seal, but also good for writing on). Regenerated cellulose is cellulose precipitated out of solution. Cellophane is clear cellulose regenerated from a viscose solution.

53.5.4 Paperboard

Waste paper can be used to produce board for cartons. Some types are

1. Chipboard: waste paper blended with wood pulp to give a flexible gray board (not suited for printing).
2. Manila-lined board: a top liner of ground wood pulp covering newspaper or other waste paper pulp, which can be printed on.
3. Clay-coated board: same as manila-lined, except that the top-liner is coated with white mineral powder bonded to the surface. This important innovation, coupled with fast electrostatic printing, has allowed the carton to be an attractive way of presenting goods.

Waste paper may also be turned into paper pulp for molding, e.g., in egg cartons, molded trays, and vegetable holders. Recycled paper is weak and discolored, making it poor for packaging. It must be used in conjunction with virgin paper. Recycled cardboard does not crease accurately, so boxes cannot be erected as accurately or quickly.

53.6 CERAMICS

The term ceramic describes any nonmetal nonorganic material produced by high temperatures, such as glass and pottery.

The raw material is molded into the required shape and then fired. Once fired, the material cannot be easily modified, as it is brittle and inert. If the material has been applied in a thin coat to another substance before firing, it is called a glaze. The most common use of ceramics in the food industry is, of course, pottery.

The chemical composition of most ceramics is silica (SiO_2), alumina ($A1_2O_3$), and water. Glass is almost pure silica, whereas clays have large amounts of alumina present. The main chemical structure of the fired product is the tetrahedral SiO_4 complex, although other stable structures like this may be present. Most clays are reddish-brown due to the presence of iron, the exception being kaolin (China clay).

During firing the clay shrinks as water is removed. Thus, control of the amount of water in the clay is important, and an initial drying stage is necessary to remove unbound moisture before firing. The firing temperature is also critical. For pottery, it should be above 1000°C. This is well below the melting point of the clay, but is high enough to cause the clay structure to break down into $A1_2O_3$ and SiO_2 molecules, which then react exothermally (sintering). A glass results from complete melting and then cooling.

Vitreous enamel is a finish applied to metals. This is done by coating the metal in powdered glass, and then heating above the melting point of the glass. Legislation nowadays prevents the use of cadmium or lead in vitreous glazes.

53.7 METALLIZED FILMS

Plastic films can be formed by lamination, coextrusion, or impregnation. No single film can satisfy all packaging requirements. Lamination is a technique for bonding films together to give a film with the properties of multiple constituents. By combining the qualities of choice from the raw material films, a laminate can be tailor-made for its particular application. Each layer in the resulting laminate may exhibit different properties from its free state, such as mutual layer reinforcement in which cracks in a brittle layer are prevented from propagating by a high elongation (elastic) layer. The effect depends on good adhesion between the layers [3]. Coatings are often used to enhance plastic film properties such as printability. Aqueous and solvent coatings are applied to the substrate through water dispersions or emulsions, solvent solutions, or waxing.

Aluminum metallized films are extensively used in food packaging applications and compared with films containing aluminum foil. Metallization has the following advantages: (i) lower environmental impact due to a significant reduction in the amount of raw material used and the recyclability of metallized film scrap as part of the base material, (ii) greater flexibility and resistance to flexion, and (iii) impressive presentation. In contrast with polymeric films, the main disadvantage of metallization is its low resistance to flexion and extension [6]. At present there are two other major limitations: foods cannot be cooked in a microwave oven within the package, and the opacity of the finished films can be a handicap for some products in which appearance is as important as quality. Thus, packaging manufacturers are looking for good barrier characteristics without compromising transparency to light and microwaves. Transparent films with excellent barrier properties have been achieved by coatings of aluminum oxide or silicon oxide [6]. Silicon-oxide-coated film helps in extending product shelf life by maximizing flavor, color, and vitamin C retention, and permitting higher filling temperatures.

53.8 BIOPOLYMERS

Bio-based materials are usually termed "environmentally friendly," 'biodegradable," or "earth-friendly." Biodegradable plastics, under appropriate conditions of moisture, temperature, and oxygen availability, lead to fragmentation or disintegration of the plastics with no toxic or environmentally harmful residue [8]. Biodegradable polymers can be classified according to their source as given in Table 53.6 [9]. The production of microbial biodegradable polymers from agro-food waste residues seems a promising route to create an innovative, more resilient, and productive waste-based food packaging bioeconomy [10].

Europe is far ahead of the United States, accounting for nearly 60% of the market for bio-based packaging [11]. Biopolymers are based on renewable raw materials, which can be processed by established plastic processing technologies such as injection and blow molding, blown or cast film extrusion, and extrusion. Components, e.g., cellulose, starch, or oils from plant-based biomaterials, such as corn, rapeseed, and soybean, can be extracted. Similarly, gelatin from animal skin and whey protein from milk can also be extracted [12]. The compostable polymers are biodegraded in an industrial composting environment in fewer than 180 days. It means a defined temperature of about 60°C, a defined humidity, and the presence of microorganisms. These do not leave fragments longer than approximately 12 weeks in the residue, and do not contain metals or toxins, and do support plant life [12]. One of the recently developed ones is compostable polymer polylactide (polylactic acid, PLA), which is typically manufactured by starch from corn. Polylactides are water-resistant

TABLE 53.6
Groups of Biopolymers

Group 1	Extracted polymers	Directly extracted or removed from biomass (i.e., polysaccharides, proteins, polypeptides, polynucleotides)
Group 2	Synthesized polymers	Produced by classical chemical synthesis using renewable bio-based monomers or mixed sources of biomass and petroleum (i.e., polylactic acid or biopolyester)
Group 3	Microbiologically transformed polymers	Produced by microorganisms or genetically modified bacteria (polyhydroxybutyrate, bacterial cellulose, xanthan, curdian, pullan)

Source: Adapted from Sorrentino et al. [9].

and can be formed by injection molding, blowing, and vacuum forming, and at room temperature. An antioxidant agent can be used in the development of biodegradable active packaging from PLA polymer matrix [13].

Polyalkylene carbonate copolymers are a unique family of innovative thermoplastics representing a true breakthrough in polymer technology. These materials are produced through the copolymerization of carbon dioxide with one or more epoxides, and these are amorphous, clear, readily processed, and long-term mechanical stability. In addition, these are considered more environmentally friendly since they consume 50% fewer petrochemicals, as compared to 100% petrochemical-based polymers, and exhibit biodegradable properties [11].

Polypropylene carbonate (PPC) is biodegradable aliphatic polyester reinforced with starch [14], and mango puree nanocomposite reinforced with cellulose nanofibers [15]. Incorporation of low-cost and biodegradable corn starch into PPC provides a practical way to produce a completely biodegradable and cost-competitive composite with good mechanical properties [14]. Improvement in the thermal properties of PPC has been reported by mixing PPC with montmorillonite clay to form a bionanocomposite [16]. Keratin films plasticized by 1,8-octanediol can be applied in food packaging and biomedical materials [17]. Keratin from duck feather without a plasticizer produces fragile films, thus formaldehyde is used for cross-linking and plasticizers to modify keratin films.

The barrier properties of bio-based packaging are crucial in terms of food packaging. Vartiainen et al. [18] reviewed the state of the art of several biopolymers including pectin, starch, chitosan, xylan, galactoglucomannan, lignin, and cellulose nanofibrils. They pointed that in most cases the packaging-related properties of single-layer biopolymer films are inadequate, and thin film coatings, such as sol-gel and atomic layer deposition (ALD), as well as multilayer coatings are applied. Peelman et al. [19] also reviewed different types of biodegradable packaging materials (such as PLA, starch, polyhydroxyalkanoates [PHA], cellulose, and polyhydroxybutyrates [PHB]) and state-of-the-art coating, nanotechnology, and physical and chemical modifications with blending. Optimum formulation was determined for the cassava starch/poly(butylene adipate-co-terephthalate) (PBAT) blown films (i.e., for biodegradable plastic bags) produced via one one-step reactive extrusion using tartaric acid (TA) as a compatibilizer [20]. It measured the tensile strength, perforation force, elongation, and seal strength.

53.9 NANOCOMPOSITES

Nanotechnology presents the packaging industry with opportunities to develop an array of exciting new products with enhanced or fundamentally different performance properties [11, 21]. In addition, it exhibits biocompatibility and biodegradability, and in some cases controlled release capacity of antimicrobial and antioxidant in the cases of active antimicrobial food packaging. Nanocomposites contain a naturally occurring polymer (biopolymer) in combination with an inorganic moiety to be functionalized, and including at least one dimension on the nanometer scale [22]. Challenges remain in increasing the compatibility between clays and polymers and reaching complete dispersion of nanoplates (exfoliation).

The most promising nanoscale size fillers are montmorillonite and kaolinite clays, and crumpled graphite nanosheets [23]. Cellulose biofibers with their highly crystalline building nanoblocks and food contact complying non-MMT (non-montmorillonite) nanoclays have been studied [24]. The reviewed papers indicated that these nanocomposites showed improved physical properties, for example, mechanical strength, thermal stability, gas barrier, physicochemical properties, and recyclability [9, 25]. Azeredo et al. [15] described the use of cellulose nanofibers and glycerol as a plasticizer to improve the mechanical and water vapor barrier properties of edible chitosan films.

In general, there is a need to develop more compatible filler–polymer systems, better processing technologies, and a systems approach to design of polymers, plasticizers, and fillers [21]. Morris [26] has expressed concerns over the long-term fate and disposal of these materials, which might then lead to release of nanoparticles into the environment and back into the food chain, raising debate on the labeling, approval, traceability, and regulation of all these smart or intelligent biomaterials materials.

53.10 CONCLUSION

Conventional packaging materials are glass, metals, paper, and synthetic polymers. Huge expansions of developing new packaging materials are progressing that consider economic, functionality, and environmental issues. These are biodegradable, green, bioactive, nanocomposites, antimicrobial, and edible packaging. In the future, significant progress in these materials will evolve.

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54 Food–Packaging Interactions

Shyam S. Sablani, Kanishka Bhunia, and Mohammad Shafiur Rahman

CONTENTS

54.1 INTRODUCTION

Packaging materials are used to protect foods against microbiological, chemical, and physical contamination during storage and distribution. However, most food packaging materials are not inert, and therefore reactions may occur between the food and packaging material. This may change the initial mechanical and barrier properties as well as safety of the packaged foods. Components of the packaging material must be safe to the product as well as to the consumers. This interaction may be classified into three main phenomena: migration, permeation, and absorption. In packaging regulations migration describes the transfer of packaging components from the packaging to the interior of foods. A distinction is usually made between global migration and specific migration. Global migration refers to the total transfer, i.e., the quantity of all substances migrating from the package into the packaged food, whereas specific migration relates to the transfer of one or more identifiable substances contained in the packaging material [1]. When volatile compounds, such as flavors and aromas, are lost through permeation and absorption, food quality may be affected. The permeation in polymeric film depends on the solution and transport behavior of gas and vapor and properties of polymers as well. Packaging materials can also absorb flavor compounds from products, reducing the desired consumer perception of quality.

Although packaging materials are necessary, they can pose risks to human health and/or affect the quality of the contained food. The quality loss of food products can occur with package failure and/or product–package interactions. Package failure can result from inadequate barrier properties for the intended shelf life or from loss of integrity during distribution [2]. Improper use or selection of packaging materials can increase the risks associated with packaged food products. For example, package failure due to loss of integrity

can greatly increase the risk of microbial contamination and potential food poisoning.

Migration of potentially toxic substances from packaging materials in the contact phase is also a major concern in the selection and use of materials for food packaging due to potential effects on human health. The migration of other components from packaging materials, while not harmful to human health, may reduce the quality of food products [2]. The sorption of fats or organic acids by the food-contact layer in a polymer laminate can cause separation (delamination) of different layers of the laminate. Other sorbed compounds can swell the polymer and act as plasticizers, thereby increasing diffusivity and permeability [3].

Product–package interactions can occur in several ways. Figure 54.1 shows a complete scenario. In some cases, product components may penetrate the structure of the packaging material, causing loss of barrier and/or mechanical properties. The migration of low-molecular-weight components from the packaging material to a contained product can result in flavor loss and/or color change. Packaging materials undergoing oxidation can also accelerate the oxidation of products in contact with that material. Packaging can absorb flavor compounds from products, the loss of which decreases the perception of quality. This scalping of flavor compounds is a concern for many aseptic products such as citrus, which contains volatile, highly aromatic compounds. When these compounds are selectively removed by the packaging material, they no longer function as flavor components. Thus, the perceived quality of the product is diminished due to loss of aroma intensity or development of an unbalanced flavor profile [3, 4]. Another problem associated with absorption of flavor components by packaging materials is the influence of that absorbent on the barrier characteristics of the packaging material. Relatively minor increases in the concentration of the organic vapor lead to large increases in permeability of the packaging material. Food-contact material may cause autocatalyzed oxidative reactions in products.

54.2 MIGRATION OF PACKAGE COMPONENTS

54.2.1 Migrating Substances

Substances that usually migrate from polymer-based packaging materials to food are plastic additives, monomers, oligomers, and contaminates. To improve manufacturability, a wide variety of additives is used during the processing of polymeric-based packaging materials. This includes plasticizers, antioxidants, light stabilizers, thermal stabilizers, lubricants, antistatic agents, slip additives, nanoparticles, and photoinitiators. This also includes migrating solvents, such as adipic acid, toluene, butanone-2, ethyl acetate, and hexane, and pigments such as molybdate orange are also used [5, 6].

54.2.1.1 Plasticizers

Plasticizers are a group of additives used in plastic materials to improve their flexibility, stretchability, and workability. The plasticizer also gives the material the limp and tacky qualities found in "cling" films. For example, a commonly used plasticizer for PVDC-based cling-films is acetyltributyl citrate (ATBC). Butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP), dicyclohexyl phthalate (DCHP), di (2-ethyl) hexyl phthalate (DEHP), diheptyl adipate (DHA), heptyl adipate

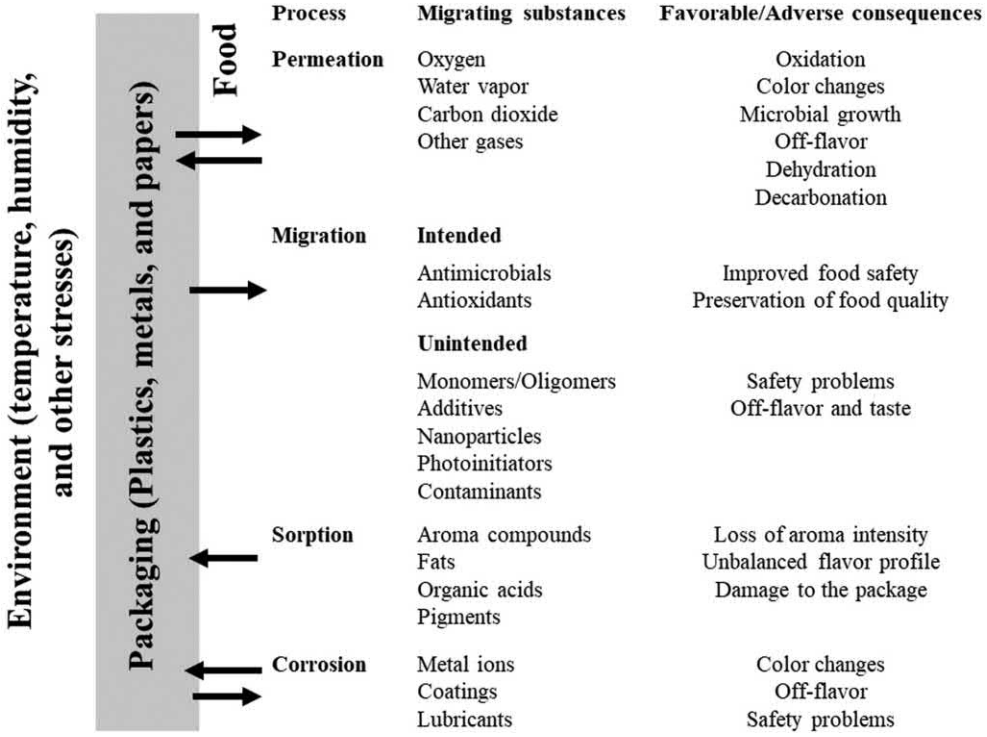

FIGURE 54.1 A complete scenario of product–package interaction resulting from several modes.

TABLE 54.1
Type and Concentration of Plasticizers Found in Some Packaging Materials

Packaging Material	Thickness (cm)	Plasticizer	Concentration of Plasticizer (μg/dm²)
Cellulose acetate	0.003	BBP	1250
		DBP	1840
		DCHP	2090
		DEHP	1620
Cellulose acetate	0.004	BBP	2050
		DBP	3170
		DCHP	3340
Cellulose acetate	0.004	DBP	4840
		DCHP	6480
Polyvinylchloride copolymer	0.002	DHA	2240
		HAD	2550
		HOA	2680

Source: Adapted from Lau and Wong [29].
BBP, butyl benzyl phthalate; DBP, di-n-butyl phthalate; DCHP, dicyclohexyl phthalate; DEHP, di(2-ethyl)hexyl phthalate; DHA, diheptyl adipate; HAD, heptyl adipate and HOA, heptyl octyl adipate

(HAD), and heptyl octyl adipate (HOA) are types of plasticizers commonly used (Table 54.1). About 80% of all plasticizers are used in PVC. Typically, phthalic esters such as dioctyl phthalate (DOP), also known as di-2-ethylhexylphthalate (DEHP), and dioctyl adipate (DOA) or di-2-ethylhexyladipate (DEHA) are used. Epoxidized soybean oil (ESBO) and low-molecular-weight polyesters are also used as plasticizers. PVC may contain 25–45% (w/w) ESBO when used as plasticizer [7]. To address the potential migration of plasticizers into food, the packaging industry has replaced PVC with other polymers, such as PE or regenerated cellulose [5]. In general, plasticizers such as polyadipates migrate at a lower rate due to their higher molar mass (1700–3400 g mol^{-1}) than low-molecular-weight plasticizers including ATBC, DOA, and dibutyl sebacate (DBS) [7–9].

54.2.1.2 Thermal Stabilizers

Thermal stabilizers are used in plastics to slow the decomposition of resins at elevated temperatures, which occurs during thermal processing. Epoxidized seed and vegetable oils such as soybean oil (ESBO), linseed oil, and sunflower oil are used in a range of food-contact plastics as heat stabilizers, lubricants, and plasticizers. Materials such as poly (vinyl chloride), poly (vinylidene) chloride, and polystyrene frequently contain epoxidized oil at levels ranging from 0.1 to 27%. Their toxicity is strongly affected by their purity, since the residual ethylene oxide is quite toxic [6].

54.2.1.3 Slip Additives and Surface Property Modifiers

Polymers such as PVC, polyolefins, and polystyrene tend to stick to metal parts during conversions due to the generation of static electricity on polymer surfaces due to friction. The addition of slip agents (antistatic) such as fatty acid esters and amides, polyethylene waxes, metallic stearates such as zinc stearate, and paraffin can prevent films from sticking together or forming conglomerates, and also reduce the accumulation of electrostatic charge [6, 10]. Slip additives are added to plastic formulations and gradually emerge and bloom on the surface. They also impart useful properties such as lubrication, better mold release, and decreased melt viscosity. Many packaging films tend to stick together because they are nonconductors of electricity. This blocking tendency can be reduced by adding anti-blocking agents such as organic amides as erucamide or metallic soaps such as zinc stearate. In some food packaging applications, moisture condenses as droplets and obstructs the view of contents. This is because polymer has a lower surface tension than water does. This prevents the formation of a continuous layer of water. Adding nonionic ethoxylates or hydrophilic fatty acid esters such as sorbitol and glycerol stearate, also known as antifogging agent, increases the critical surface tension of the polymer surface and promotes the deposition of continuous films of moisture.

54.2.1.4 Anti-Aging Additives

Aging is the process of the deterioration of materials, which results from the combined effects of atmospheric radiation, temperature, oxygen, water, microorganisms, and other atmospheric agents (e.g., gases). Antioxidants are used to slow down oxidation of plastics due to light exposure. BHT, BHA, Irganox 1010, Cyanox 2246, bisphenol A, and Irgafos 168 are the most commonly used antioxidants. Antimicrobials such as algaecides, bactericides, and fungicides can be added to polymers to prevent the growth of microorganisms. UV stabilizers are used to prevent deterioration of polymeric films by photo-oxidation. They act by absorbing high-energy UV radiation and releasing it as lower energy radiation [11].

54.2.1.5 Optical Property Modifiers

Optical property modifiers are used to modify the ability of plastic to transmit light, exhibit color, and reflect surface light (i.e., gloss). Some of the pigments for use as colorants in packaging are red iron oxide, carbon black, white titanium dioxide, yellow cadmium sulfide, ultramarine blue, blue ferric ammonium ferrocyanide, blue and green copper phthalocyanines, molybdate orange, and chrome green. However, a major number of food packaging films do not go through pigmentation. Due to their potential to migrate from plastics into food products, the FDA has questioned the use of some these colorants [10].

54.2.1.6 Monomers and Oligomers

Monomers and oligomers can easily migrate from packaging material into food [5, 6]. Monomers are reactive substances and potentially toxic to living organisms. Therefore, hygiene regulations aim at restricting the content of residual monomers in raw materials and plastics.

Unreacted residual styrene in PS food packaging can migrate into food and may cause adverse and harmful effects on human health. Vinyl chloride monomer is highly toxic;

thus, levels of PVC in food packaging materials are tightly controlled. The epoxy resin of bisphenol A type, such as bisphenol A diglyceride ether (BADGE), is the main component of internal can linings. BADGE has cytotoxic effects in living tissues, and may cause a higher rate of cell division [6]. It may contain unreacted BPA, which is also a major component of polycarbonate-based beverage bottles. However, the FDA found that the current level of BPA/BPA-based materials is safe for food-contact applications, with the exception of baby bottles, sippy cups, and infant formula packaging [12].

Other compounds such as isocyanates used in polyurethane polymers and adhesives are toxic, with well-documented health effects. Polyethylene terephthalate (PET) is commonly used as a packaging material for beverages and edible oils. A standard limit has also been established for the migration of oligomers from PET packaging. Nylon, a frequently used polymer for thermal processing, releases nylon-6 oligomers and caprolactam (monomer) during various cooking methods [13, 14]. These compounds can migrate from polyamides into boiling water and impart a bitter taste to foods.

54.2.1.7 Contaminants

Additional sources of food contamination include decomposed products from additives and monomers that may also migrate into foods. Some of these compounds are diphenylthiurea, benzene dioxins, hydrogen peroxide, and other volatiles [5]. Food can also be contaminated by hydrocarbons, including mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) [15]. MOAH is carcinogenic and mutagenic, as well as an endocrine disruptor. Accumulation of MOSH in the human body can cause dysfunction. Potential sources of such contamination are recycled paperboard used as packaging for dry foodstuffs such as noodles, rice, and bakery foods, as well as mineral-oil-based printing inks [16].

54.2.1.8 Nanoparticles and Coating Substances

Nanoparticles are incorporated into polymeric packaging to improve the properties of packaging polymers, such as mechanical strength and barrier properties against oxygen, water vapor, carbon dioxide, and volatiles. Aluminum (Al) and silicon (Si) metal oxide coatings are used to develop high gas barrier and high-performance polymeric packaging for thermal and nonthermal processing technologies. Functional nanoparticles (Ag, ZnO, TiO_2, Se, Cu) with antimicrobial or antioxidant activities are often used in polymeric packaging to increase the shelf life of food. However, nanoparticles and metal from coatings can migrate from food packaging into food and pose risks to human health [17–19]. Nano-silver that is incorporated in food containers and polymer coatings exhibits varying degrees of migration, depending on the type of packaging and food used [19, 20].

54.2.1.9 Photoinitiators

Photoinitiators are a mixture of monomers, oligomers, pigments, and photoactive compounds used in the formulation of UV-curing printing inks, as well as varnishes for food packaging polymers. They can also be found in recycled paper packaging and printed secondary packaging. Photoinitiators such as Irgacure, benzophenone, and isopropylthioxanthone (ITX) can contaminate foodstuff by mass transference [21–23]. The migration of photoinitiators occurs through two mechanisms: set off and/or permeation migration [23]. During "set off," photoinitiators from the external printed surface are transferred to the internal non-printed layer due to contact. Permeation migration occurs when photoinitiators from the external printed surface reach the internal food-contact layer through substrate. Semi-volatile photoinitiators such as benzophenone and its derivatives can contaminate food indirectly through vapor [22].

54.2.2 Prediction of Migration

Migration is a complex process in which substances are diffused from the food-contact layer (higher concentration zone) to the food surface (lower concentration zone). This is dependent on several factors, including the nature of the food, temperature, and the duration of storage [24]. The amount of migration into food can be predicted using a mathematical model based on diffusion. Mathematical models are useful tools for the identification of factors affecting migration and in package design. A better understanding of the migration process will help control the chemical contamination of food from packaging [5]. The diffusion of migrants in polymer and food can be described using Fick's second law:

$$D_p \frac{\partial^2 C_p}{\partial x^2} = \frac{\partial C_p}{\partial t} \tag{54.1}$$

$$D_s \frac{\partial^2 C_s}{\partial x^2} = \frac{\partial C_s}{\partial t} \tag{54.2}$$

where D_p and D_s are the diffusivity of migrant in polymer and food phase (m^2/s); C_p and C_s are the concentration of the migrant in the polymer and food phase (mg/g); x is the space coordinate measured normal to the polymer-food interface (m); and t is time (s). The amount of package components that may migrate into liquid or solid food depends on the chemical and physical properties of the food and packaging. Various factors such as migrant concentration, molecular weight, solubility, diffusivity, partitioning coefficient between polymer and food, time, temperature, polymer and food composition, and structures (density, crystallinity, and chain branching) control migration.

Several semi-empirical diffusion-based mathematical models have been developed and suggested (Table 54.2). Briston and Katan [25] divided migration into three classes based on the limiting control mechanism. Class 1: non-migrating materials with or without the presence of food, Class 2: independent migration, which is not controlled by the food, although the presence of food may accelerate it, and Class 3: leaching, which is controlled by the food, negligible in the absence of food, and significant in its presence. There is

no absolute cut-off between these classes, and the definitions of words like "significant" can influence where the cut-offs are placed [26]. Class 1 is applied for diffusivity of less than 10^{-15} m^2/s. On the other hand, Class 3 systems are those with diffusivity 10^{-13} m^2/s or higher in the presence of food. The migration process is fully described by the kinetics of migrant diffusion in each phase, expressed by diffusivity (D) and the chemical equilibrium, expressed as partition coefficient, K, defined as the ratio of migrant equilibrium concentration in the polymeric material, C_p, to its equilibrium concentration, in the food phase, C_s. K is defined as:

$$K = \frac{C_p}{C_s} \tag{54.3}$$

when $K = 1$, the migrant concentration in food phase equals the concentration in the polymeric phase, at equilibrium. K is higher when more migrant is absorbed into the polymer than into the food. In terms of food safety, a large K limits the migration from packaging material to food. On the other hand, a low K indicates that more migrant is absorbed into the food from the polymer. The food-contact layer is usually made of non-polar polyethylene (PE) or polypropylene (PP), and as a consequence, $K \leq 1$ between the polymer and organic solvents or fatty foods. As the polarity of food increases, the K value also increases. The K value of pure water is over 1000, which eventually limits the migration process. Therefore, K values of 1 and 1000, on either side of the spectrum, are considered as the "worst case" for evaluation of food safety [27].

The partition coefficient is generally estimated by either of three following approaches [27]: the regular solution theory (RST), the unified quasi chemical theory of liquid mixtures functional-group activity coefficients (UNIFAC), and the retention index system. However, to minimize flavor loss in a package, a low K is preferred. Parameters such as the temperature, pH, chemical structure of migrant, molecular size and structure, degree of crystallinity, and polarity of solutes, plastics, and the food can all influence the partition coefficient [27, 28]. Lau and Wong [29] found K values from 2.5 to 250 and D_s from 1.1×10^{-16} to 2.7×10^{-15} m^2/s for three different plasticizers/food systems, with much larger D_s values in fatty foods. Nielsen et al. [30] estimated a K value of up to 22 for four aroma compounds in a water/LDPE system under different storage temperatures.

Several user-friendly software tools, such as MIGRATEST© Lite (FABES GmbH, Munich, Germany), AKTS-SML (AKTS, AG, Switzerland), and MULTITEMP and MULTIWISE (INRA, Reims, France), have been developed for performing migration analysis. MIGRATEST© Lite is based on the Piringer model (Table 54.2), which employs an analytical solution of transport equations to estimate the diffusion coefficients whereas AKTS—SML uses a finite element approach to solve the transient Fick's second law.

54.2.3 Migration Testing and Analytical Methods

Analysis of the migrant in foods can be expensive and time-consuming. This is due to low concentrations of migrated substances and the complexity of the food matrix. Sophisticated analytical methods and protocols have been developed to study the migration of packaging components from packaging or food-contacting material into the food. These procedures are not only important for quantification of migrants, but are also required in order to establish databases for evaluating changing residue levels. They are also used to calculate dietary intakes. These methods and protocols have been approved by regulatory agencies such as the FDA and EC. The analytical procedures involve sample preparation, extraction, cleanup, and a final determination using chromatographic or spectrophotometric analysis.

Various analytical instruments, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and GC–mass spectrometry (GC–MS), have been used for routine analysis. For example, HPLC methods have been used to measure Bisphenol-A in epoxy resins and diglycidyl ether of DGBPA in tin and 4,4-bis-(dimethylamino benzophenone) in paper and board. GC and GC–MS are used to measure 4,4-bis-(diethylamino benzophenone) in paper and board, styrene dimmers/trimmer in polystyrene cups, and mineral hydrocarbons in polystyrene and wax paperboard. Advanced instruments such as liquid chromatography–tandem mass spectrometry (LC–MS/MS), LC with fluorescence detection, 3D-front face fluorescence, ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC–qTOF-MS/MS), and inductively coupled plasma mass spectrometry (ICP-MS) are also used to detect and quantify additive(s)/mixture of additives in complex biological materials. Independent component analysis (ICA) has been used to analyze 3D-front face fluorescence spectra of olive oil to analyze packaging–oil interaction [31]. The migration of nano-silvers and silicon from polymer coatings into foods/food simulants has been quantified using ICP-MS [17, 20]. A Fourier transform infrared spectroscopy (FTIR) method has proven useful for analyzing substances on polymer and internal can coatings. Due to the complex nature of foods and the variety of conditions arising from contact with packaging, a series of steps is necessary to quantify specific and overall migration. To simplify the process, regulatory agencies have approved the use of food simulants (Tables 54.3 and 54.4) for migration studies.

54.2.4 Factors Affecting Migration

54.2.4.1 Glass Transition Temperature of Polymers

For contaminants from the surrounding environment, such as naphthalene or other volatile organics, the rate of migration is affected by the glass transition temperature (T_g) of the polymer. In the glassy state (below T_g), polymer molecules/chains are rigid, and migrant molecules have less free volume to diffuse through. This results in a slower migration rate. Above T_g, polymer is flexible due to increased mobility of segmental chains, allowing a higher rate of molecular movement of migrants. Additionally, glassy polymer consists of nanovoids that provide strong adsorption sites to migrating molecules and retard the release rate of migrating compounds [32]. At room temperature,

TABLE 54.2
Selected Mathematical Models Proposed for Description of Migration of Substances

Model	Reference
$M_t = 2C_0\rho\left(\frac{D_p t}{\pi}\right)$	Arvanitoyannis and Bosnea [5]
M_t = total migrant from the polymer in time, t (s); C_0 = initial migrant concentration in the polymer (mg/g); ρ = polymer density (g/cm^3); D_p = diffusivity of migrant in polymer (cm^2/s), t = package life time (s); $\pi = 3.142$	
$\frac{m_{s,t}}{A} = c_{p,0}\rho_p d_p\left(\frac{\alpha}{1+\alpha}\right)\left[1-\sum_{n=1}^{\infty}\frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2}\exp\left(-D_p t\frac{q_n^2}{d_p^2}\right)\right]$	Piringer [91]
$\frac{m_{s,\infty}}{m_{p,\infty}} = \frac{\alpha}{1+\alpha},\ q_n = (2n-1)\pi/2$	
$m_{S,t}$ = amount of migrants transferred into food at time t (s); A = surface area of food-contact layer	
$m_{S,\infty}$ = amount of migrants transferred into food at equilibrium; $m_{P,\infty}$ = amount of migrant retained in polymer at equilibrium; $c_{P,0}$ = initial concentration of migrant in polymer (mg/g); ρ_P = density of polymer (g /cm^3); = thickness of packaging material; D_p = diffusivity of migrant in polymer (cm^2/s)	
$\frac{M_t}{A} = \frac{2\sqrt{D_s t}\cdot K\cdot C_0}{1+K\cdot\alpha}\left[\frac{1}{\sqrt{\pi}} - i\cdot\mathrm{erfc}\left(\frac{L}{2\sqrt{D_p t}}\right)\right]$	Lau and Wong [29]
$\alpha = \sqrt{D_s / D_p}$; K = *p*artitioning coefficient and $i\cdot\mathrm{erfc}(x) = \frac{1}{\sqrt{\pi}}\exp(-x^2) - x\cdot\mathrm{erfc}(x)$	
D_p and D_s = diffusivity of migrant in polymer and food phase (m^2/s); C_p and C_s = concentration of migrant in the polymer and food phase (mg/g); L = thickness of polymer (m); t = time (s)	
$M_t = 2C_0\left(\frac{D_p t}{\pi}\right)^{1/2}\left[\frac{\beta}{1+\beta}\right]$	Till et al. [92]
$\beta = K\left(\frac{D_s}{D_p}\right)^{1/2}$	
t = storage time; M_t = amount of migrant; K = partitioning coefficient; D_p and D_s = diffusivity of migrant in polymer and food phase; C_0 = initial migrant concentration in the polymer	
$\frac{M_t}{A} = \sqrt{D_s t}\cdot K\cdot C_0\left(1-\frac{\sqrt{D_p t}}{3L}\right)$	Lau and Wong [29]
D_p and D_s = diffusivity of migrant in polymer and food phase (m^2/s); C_p and C_s = concentration of migrant in the polymer and food phase (mg/g); L = thickness of polymer (m) t=time (s)	
Migration across recycled plastic and food film barrier	Frank et al. [93]
$M_t = \frac{2}{\sqrt{\pi}}\left[C_P\left(1+\frac{b}{d}\right) - C_B\frac{b}{d}\right]\ {}_P\sqrt{D_P}\left(\sqrt{{}_r + t} - \sqrt{{}_r}\right)$	
b = thickness of the barrier, d = thickness of the recycled plastic, C_B = the migrant concentration in the barrier layer, θ_r = lag time of the migrant across the barrier layer, and ρ_P = density of the recycled plastic layer	
$M_t = C_{\mathrm{sat}}\gamma_{\mathrm{air}}\sqrt{\frac{Dt}{\pi}}\left[1.33\beta t - 0.54(\beta t)^2 + 0.15(\beta t)^3 + \ldots\right]$	Lau and Wong [94]
β = kinetic factor, C_{sat} = saturated migrant concentration in the packaging material when exposed to saturated migrant vapor, γ_{air} = ratio of migrant concentration in air to that of the saturated migrant concentration in air	

(*Continued*)

TABLE 54.2 (CONTINUED)
Selected Mathematical Models Proposed for Description of Migration of Substances

Model	Reference
$C_{F,t} = \frac{A}{m_F}\rho_P C_{P0}\sqrt{D_P t}$	Baner et al. [95]
$C_{F,t}$ = estimated concentration, ρ_P = density of the plastic material, D_P = migrant diffusivity and (A/m_F) = package surface area, $D_P = 10^4\exp(A_P - aM_r - bT^{-1})$ where the coefficient A_P accounts for the effect of the polymer on diffusivity (A_p values for different polymers are 9 for LDPE, 5 for PP and HDPE, ≤0 for non-polyolefins, –7 for PVC, and –3 for PC and PET), M_r = substance relative molecular weight, T = temperature (K), and a and b are correlation constants for molecular weight and temperature effects on diffusivity bearing values of 0.010 and 10450, respectively	
Weibull model $\frac{C_t - C_\infty}{C_0 - C_\infty} = \exp\left[-\left(\frac{t}{\tau}\right)^\beta\right]$	Poças et al. [96]
C_t is the migrant concentration in food at any time t, C_∞ is the concentration at equilibrium, C_0 is the initial concentration, τ is scale parameter associated with process rate, and β is the shape parameter or behavioral index	

polymers such as polyethylene and polypropylene with T_g lower than room temperature have a larger permeability for organic compounds than those with a T_g higher than room temperature, such as polystyrene and PVC. The melting of polymers significantly increases the release of substances from polymers.

54.2.4.2 Molecular Weight and Geometry of Migrants

Migrant molecules with different shapes, sizes, volumes, and weights influence diffusion in polymers, and therefore control the migration process. Substances with higher molecular weight and volume hinder the diffusion process. The volume of molecules, which combines the shape factor and the fractionated volume, correlates with the diffusivities of migrants. For example, the diffusivity of Irganox 1330 in PP at 40°C is two orders of magnitude less than that of Uvitex OB because the Irganox molecule is 1.7 times larger than Uvitex OB [33]. The diffusivity of limonene in PP at 40°C is almost four orders of magnitude higher than Irganox 1010 due to limonene's smaller size and molecular mass (M_r = 136 g/mol) compared to Irganox 1010 (M_r = 1178 g/mol) [34]. Generally, there is a linear relationship between $\log(D_p)$ and molecular weight, and the weighted fractionated volume of molecules [33]. Larger nanoparticles also demonstrate slower diffusion from LDPE film (Table 54.5) [35].

TABLE 54.3
List of Food-Simulating Solvents for Migration Testing, as Defined in the Code of Federal Regulations

Type of Food	Recommended Simulants
Aqueous and acidic foods (types I, II, IVB, VIB, and VIIB)	10% ethanol
Low and high alcoholic foods (types VIA, VIC)	10 or 50% ethanol
Fatty foods (types III, IVA, V, VIIA, IX)	Food oil (e.g., corn oil), HB307, Miglyol 812, or others

Source: 21CFR 176.170(c) [90].
Note: HB307 is a mixture of synthetic triglycerides, primarily C_{10}, C_{12}, and C_{14}.

54.2.4.3 Solubility of Migrants at the Polymer–Food Interface

A migrant with greater solubility into food has a smooth and continuous concentration profile at the polymer–food interface. This facilitates the rate of migration into the food. On the other

TABLE 54.4
Types of Raw and Processed Foods

Food Category	Description
Type I	Nonacid, aqueous products; may contain salt or sugar or both (pH above 5.0)
Type II	Acid, aqueous products; may contain salt or sugar or both, and including oil-in-water emulsions of low- or high-fat content.
Type III	Aqueous, acid or nonacid products containing free oil or fat; may contain salt, and including water-in-oil emulsions of low- or high-fat content
Type IV	Dairy products and modifications: (A) water-in-oil emulsions, high- or low-fat and (B) oil-in-water emulsions, high- or low-fat
Type V	Low-moisture fats and oil
Type VI	Beverages: (A) containing up to 8% alcohol; (B) nonalcoholic; (C) containing more than 8% alcohol
Type VII	Bakery products other than those included in type VIII or IX: (A) moist bakery products with surface containing free fat or oil; (B) moist bakery products with surface containing no free fat or oil
Type VIII	Dry solids with the surface containing no free fat or oil (no end test required)
Type IX	Dry solids with the surface containing free fat or oil

Source: 21CFR 176.170(c) [90].

TABLE 54.5
Diffusion Coefficient of Selected Chemical Substances

Category	Specific Migrant	Packaging Material	Food/Solvent	T, °C	D_p, cm²/s	Reference
Plasticizer	DMP	Paper	Tenax	23	7.29×10^{-10}	Pocas et al. [96]
	DEP				4.66×10^{-10}	
	DBP				3.87×10^{-10}	
	DHP				1.91×10^{-10}	
	DOP				1.53×10^{-11}	
Antioxidant	Irgafos 168	LDPE film	Tenax	23–70	4.8×10^{-13}–7.27×10^{-12}	Reinas et al. [97]
			Rice	23–70	9.57×10^{-18}–4.33×10^{-17}	
	Irganox 1076	LDPE film	Tenax	23–70	8.34×10^{-13}–1.95×10^{-11}	
			Rice	23–70	6.9×10^{-18}–3.71×10^{-17}	
Additive	DPBD	LDPE film	Minced pork	5–25	1.2×10^{-10}–1.88×10^{-9}	Silva et al. [98]
			Pork neck	5–25	6.41×10^{-12}–5.41×10^{-11}	
			Chicken	5–25	1.3×10^{-12}–2.86×10^{-12}	
			Margarine (61% fat)	5–25	4.2×10^{-10}–5.1×10^{-9}	Silva et al. [99]
			Margarine (80% fat)	5–70	3.0×10^{-10}–2.7×10^{-8}	
			Chocolate (32% fat)	25–70	2.9×10^{-10}–1.5×10^{-8}	
			Chocolate spread	5	9.1×10^{-10}	
			Soft cheese	5	3.2×10^{-11}	Cruz et al. [10]
			Cottage cheese	5	1.12×10^{-9}	
			Gouda cheese	5	6.34×10^{-11}	
Antimicrobial	Triclosan	Nylon control film	10% ethanol	25–70	5.79×10^{-13}–3.52×10^{-9}	De Abreu et al. [101]
			95% ethanol	40	1.88×10^{-10}	
			Isooctane	60	5.36×10^{-11}	
		Nylon nanocomposite film	10% ethanol	25–70	3.23×10^{-13}–5.54×10^{-10}	
			95% ethanol	40	1.02×10^{-10}	
			Isooctane	60	2.21×10^{-11}	
Monomer	Caprolactam	Nylon control film	10% ethanol	25–70	9.98×10^{-12}–1.02×10^{-9}	De Abreu et al. [101]
			95% ethanol	40	8.82×10^{-11}	
		Nylon nanocomposite film	10% ethanol	25–70	5.89×10^{-12}–5.10×10^{-10}	
			95% ethanol	40	7.05×10^{-11}	
Monomer	Laurolactam	Nylon 12	Isooctane	40–80	3.6×10^{-12}–3.0×10^{-10}	Stoffers et al. [102]
			Olive oil	40–80	1.1×10^{-12}–3.4×10^{-11}	
Monomer	Styrene	PS cup	Cooking oil	70–150	8.8×10^{-11}–2.6×10^{-9}	Lickly et al. [103]
		Plate	Cooking oil	70–150	1.0×10^{-11}–2.5×10^{-9}	
		Meat tray	Cooking oil	70–150	4.6×10^{-11}–4.2×10^{-9}	
Low-molecular-weight compounds	Acetaldehyde	PET	Carbonated mineral water	23–50	4.4×10^{-12}–4.59×10^{-11}	Welle and Franz [104]
	Benzene	PET	Carbonated mineral water	23–50	3.11×10^{-12}–5.76×10^{-14}	
Catalyst	Antimony	PET bottle for carbonated drink	Milli-Q water	25–70	3.6×10^{-21}–5.9×10^{-18}	Rungchang et al. [105]
			4% acetic acid	25–70	5.5×10^{-20}–3.8×10^{-17}	
			50% ethanol	25–70	5.5×10^{-20}–3.6×10^{-17}	
Nanoparticle	Titanium nitride (dia = 1–10 nm)	LDPE film	-	40	4.3×10^{-9}–1.1×10^{-35}	Bott et al. [35]

Note: T is the temperature at which the experiment was conducted; D_p is the diffusion coefficient of migrants in packaging.

hand, if the migrant partitions poorly into the food, the migrant concentration profile may be discontinuous at the interface, slowing the rate of migration. Since most polymer additives and contaminants are fat-soluble, contamination of food by migrating chemicals is more serious in fatty foods than in aqueous foods.

54.2.4.4 Polymer Swelling

Swelling of polymeric materials may occur when foods/food simulants such as organic solvents, lipids, or water are absorbed by polymers. This absorption may create structural changes in polymers that can increase the D_p of additives by several orders of magnitude. Reynier et al. [36] reported a one to two order of magnitude increase in D_p in swollen PP at 40°C for a wide range of additives, including Irganox and Irgafos. The swelling-dependent diffusion coefficient, $D(C)$ can be expressed in three mathematical expressions [37–39]:

$$D(C) = D_0 + B \times C \tag{54.4}$$

$$D(C) = D_0 \times \exp(B \times C) \tag{54.5}$$

$$D(C) = D_0 \times \exp\left(B \times \frac{C}{1+C}\right) \tag{54.6}$$

where D_0 is a constant, B is the "swelling constant," and C is the concentration of the substance causing swelling.

54.2.4.5 Dispersion into Bulk Food

Once the migrant molecules are solvated, they diffuse away from the interface and move into the bulk food. Migration at this stage and in the two previous stages is driven mainly by entropy, a measure of randomness. Limm and Hollifield [40] found that mixing can increase migration into food since it enhances kinetically migrant solvation by removing migrants from the interface, thus reducing re-precipitation. However, migrant solubility and diffusion coefficients are the prime factors governing the dispersion of migrants into food, thus affecting the rate of migration as whole.

54.2.4.6 Temperature

Molecular mobility increases with increasing temperature and accelerates the molecular diffusion of chemical substances (Table 54.5). Thermal processing involves higher temperatures, resulting in a higher migration rate from polymeric packaging into food. The diffusion coefficient of packaging material components can increase six- to seven-fold if the material is exposed to extreme temperature fluctuations (e.g., freezer temperatures to cooking temperature) [24]. Such increases in temperature may also cause the transition of polymers from the glassy to rubbery state, further increasing the migration rate. Brandsch et al. [41] modified a correlation to predict the diffusion coefficient of migrants as a function of the molecular mass of migrant and absolute temperature:

$$D_p = D_0 \exp\left(A_p - 0.1351 M_r^{2/3} + 0.003 M_r - 10454 / RT\right) \text{cm}^2/\text{s} \tag{54.7}$$

where D_0 is the pre-exponential factor, $A_p = A_p^{'} - \tau / T$, M_r is the relative molecular mass of the migrant, and $A_p^{'}$ and τ are specific parameters of the polymer matrix. R is the gas constant, and T is the absolute temperature.

54.3 FOOD AND PACKAGING INTERACTION

54.3.1 Metal–Food Interaction

Metal containers provide absolute protection to food from ambient conditions and environmental contamination, ensuring food safety and quality retention with long-term storage stability. In the market, metals are mainly in the form of cans and flexible pouches for shelf-stable foods, as well as containers for beverage packaging and household usage. However, metal packaging may fail due to stress and corrosion. Corrosion is the destructive attack on a metal through chemical or electrochemical reaction with the environment. Since steel corrodes rapidly in the presence of acidic substances, the tin acts as a barrier. Some cans are lacquered internally for high-acid products (pH < 3) or for products that change color in the presence of tin. Containers made of tinplate are resistant to corrosion by high-acid foods, and can be used unlacquered. However, foods that contain sulfur can cause the tin to blacken. Therefore, metal containers usually come with internal organic coatings or enamel coating. Since steel provides nearly complete barrier protection and has the mechanical strength to handle pressure, it can be retorted (cooked under pressure) after sealing [42]. Cans are susceptible to corrosion at the stressed areas, called stress corrosion cracking (SCC), as well as rapid iron dissolution at fractures/pores in the organic coatings, which causes product blackening known as pitting corrosion. They are also susceptible to sulfide black corrosion, which is a blackening of the internal part of cans and discoloration of foods, and rust formation on the external damage of the cans known as filiform corrosion. Blistering of flexible pouches containing aluminum foils may also cause contamination in foods. The chances of such failure increase considerably in the high temperatures and pressures of retort processing. Furthermore, important factors causing corrosion are enamel properties (porosity, corrosion resistance, and adhesion), the nature of the coating (polyester, epoxy resins, acrylic, oleoresins), the thickness and porosity of coatings, the nature of aqueous or fatty foods, including acidity, oxygen, nitrates, sulfur compounds, trimethylamines, anthocyanins, and dihydroascorbic acid as well as the severity of heat treatment and storage conditions [7, 43, 44].

The major corrosion products in food cans are mainly limited to three metals, tin, iron, and lead, which are liable to dissolve from the container. Of these, only the lead is toxic and cumulative in body tissues, and hence poses a hazard. Lead content varies widely within and between food products. Even the raw material may contribute some of it, although generally the level is well below the regulatory limits, which is 2 ppm for most foods, 0.5 ppm for baby foods, and 0.2 ppm for soft drinks [44]. Consumption of lead may lead to reduced

intellectual performance of children and increased cardiovascular diseases in adults. In a survey, the lead content of 168 samples of acid foods was found to be in the range of 0.02–8.16 ppm, the average for lacquered cans being 1.45 ppm and for plain cans 0.46 ppm [44]. The shift from three-piece soldered cans to three-piece welded or cemented cans, or to two-piece cans, has eliminated possible lead migration from cans to foods [44]. However, commercial tin–lead alloys used for soldering the seams of tin cans contain almost 98% lead. Thus, the possibility of lead migration into canned foods still exists [44]. The lead content in a wide variety of canned fishes has been measured below 0.20 ppm [45]. Canned infant foods are soldered with pure tin to avoid lead contamination, and welding is increasingly used instead of soldering for the same reason [44]. According to the recent FDA guidelines, maximum allowable lead contents of 0.1 ppm in candy for small children, 0.005 ppm in bottled water, and 0.05 ppm in fruit juices are safe for consumption.

Iron, an essential constituent of our diet, does not constitute a toxicity problem, and a limit of 50 ppm is usually considered acceptable. However, rapid dissolution of iron due to pitting corrosion darkens the color of canned foods, such as blackening of olives and brine [46]. Most of the tin in canned foods is insoluble in gastric and intestinal fluids, and is not absorbed during digestion. In solid foods, high levels may occur. Metallic tin and its salts are considered to have low oral toxicity, whereas alkyl derivatives are highly toxic. Outbreaks of poisoning manifested by nausea, vomiting, and other gastrointestinal disturbances have been traced to solid foods and drinks containing high levels of tin (hundreds of ppm). The 250-ppm level quoted in the past as a permissible upper limit in canned foods is based not on toxicological evidence of safety, but on the fact that higher levels produce off-taste and are rarely found under normal conditions of processing and storage. This value is 150 ppm for canned beverages. In lacquered cans, the tin content of the food rarely exceeds 100 ppm [44]. Cans with a lacquered inner wall showed tin migration almost ten times lower than that of non-protected or partially protected cans for a wide range of fruits and vegetables [47]. Recent studies also reported the migration of other trace elements including cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr), manganese (Mn), and nickel (Ni) into foods from cans and stainless-steel containers [48–50].

Migration in metal cans is primarily associated with the transfer of compounds from solvents used for coating. Mesityl oxide, a solvent used to coat side seams, imparts a "catty" flavor defect to packaged pork products. It interacts with free sulfhydryl groups of meat proteins and trace amounts of free hydrogen sulfide present in meat. Inophorone, a contaminant of can coating solvents, causes a "chemical" defect in canned milk beverages. Epoxy coatings are responsible for anomalous flavors in canned beers [51]. Soft drinks and especially beer are very delicate products, and their flavor and clarity are easily affected if in contact with tin, iron, or an unsuitable varnish. The taste of beer, for example, is adversely affected by dissolved iron above 0.1 ppm [44]. Another major concern is the migration of BPA from epoxy coatings on internal can surfaces. Although the presence of BPA in food does not alter taste significantly, it may pose serious health concerns due to its severe toxicity above the specific migration limit. Some chemical substances that can migrate from metals into food are listed in Table 54.6.

Lubricants are normally used with tinplate to prevent abrasion and facilitate handling during conversion into containers. Typical lubricants contain fatty acids and esters prone to oxidation, and some oxidation products may be transferred to packaged food [51]. Lubricants can result in stale, rancid, woody, and cardboard-like off-flavors in canned beer [52]. Such a compound is 2-nonenal, which has a sensory threshold of about 1 ppb.

54.3.2 Paper–Food Interaction

Paper and board serve as primary, secondary, and tertiary packaging. Migration mainly occurs during primary packaging, i.e., when paper and board come directly in contact with dry foods. Nevertheless, food contamination due to the transfer of volatile and semi-volatile substances from secondary packaging may also occur. Most documented cases of migration from paperboard or paperboard/plastic laminates pertain to components transferred from solvents and adhesives used for material and/or package fabrication, or those transferred from inks used for printing [52–54]. The major migrating components from paper and board packaging are diisobutyl phthalate (DiBP), diisopropyl naphthalenes (DIPNs), di(2-ethylhexyl) phthalate (DEHP), benzophenone (BP), triclosan (TRCL), and fluorochemicals (Table 54.6).

Recycled paper and paperboard are used for many commercial applications due to environmental concerns. However, recycled paper materials contain adhesives, printing inks, trace elements, bleach, paper straightening agents, and inks. Benzophenone, a photoinitiator in UV-cured inks used for printing on cardboard and paper, may also be present in recycled papers. In one study, 17 packages containing recycled materials were analyzed for BPA, DEHP, nonylphenol monoethoxylate (NMP), and nonylphenol di-ethoxilate (NDP) and their migration into salt, sugar, and Tenax [55]. BP and ink compounds can also migrate from carton board to food during microwave heating, and even at frozen storage at –20°C. [56]. Four of the seven carton boards were found to contain BP in the range 0.4–3.0 mg/d m^2. When potato chips and hamburger were heated in a microwave after 1 year of frozen storage, nearly 17 and 8% of the BP present in the carton board migrated, respectively. In Australia, UHT milk packaged in HDPE bottles was recalled due to the metallic taint of benzaldehyde (25 ppb) and BP (10 ppb) that migrated from printing inks on the external surface of the bottles into the milk [10]. Hydrocarbon-like off-odors have been reported in doughnuts packaged with wax-coated paperboard dividers [54]. Benzophenone (BP) solvents used for printing paper have been implicated in the migration of solvent residues into packaged yogurt, resulting in a chemical taste [54]. The compounds migrated from the adhesive layer of aluminum-baked paperboard packaging have been found to cause off-flavors.

TABLE 54.6
Migrating Components from Packaging Materials (Other Than Polymers) to Food

Packaging Material	Migrating Component	Food	Reference
Wooden packaging	1-propanol	Apples	Mousavi et al., [106]
Tin	DGEBA	Canned foods	Biles et al., [107]
Metals/plastics/glass/aseptic	DIPNs	Tomato	Casp et al., [108]; UK, [109, 110]
Recycled paper and board Cans coated with lacquer	Epichlorohydrin		
Paper cardboard and board	Metals (Zn, Sn, Al, Mn, Ba)	Test foods	Knezevic, [111]
Cartons (Al-laminated)	Al	Skim milk, yogurt drink	Eklund and Brenne, [112]
Aseptic	H_2O_2	Milk	Satyanarayana and Das, [113]
Aluminum foil paper laminates	Phthalate esters (DBP, BBP, DEHP)	Butter, margarine	Page and Lacroix, [114]
Cans	BADGE (lacquer)	Water-based simulants	Paserio et al., [115]
Aluminum	Al	Food and drinks	Muller et al., [116]
Paper-based food packaging	2378-TCDD/2378-TCDE (polychlorinated dibenzofurans)	Fatty and non-fatty foods	LeFleur et al., [117]
Ceramic containers	Pb, Cd	Dairy products	Cabrera et al., [118]
Aluminum	Al	Milk	Sieber and Daniel, [119]
Cans	BADGE	Canned foods	UK, [109]
Aluminum	Al	Dairy products	Sieber and Daniel, [120]
Paper and board	4,4-bis(dimethylamino benzophenone((MK), 4,4-bis-(diethylamino benzophenone((DBAB)	Dairy products	Castle et al., [121]
Recycled paperboard, printed packaging	Photoinitiators	FSL, dry foods	Biedermann et al., [122]; Sanches-Silva et al., [23]

A variety of odor-active (low sensory threshold) phenols and cresols, such as p-chloro-m-cresol, have been identified [57]. Similarly, pentachlorophenol and methyl chloroform have been traced to adhesives used for paperboard package fabrication [52]. Pentachlorophenol is used as a biocide in certain adhesives, and causes a moldy off-flavor in food products. Moldy off-flavors due to the migration of 2,4,6 trichloroanisole into cocoa power packaged in paper board containers have also been reported [52]. The paper or paperboard manufacturing process itself can result in the formation of potential migrants. These are chlorophenols, such as 2,4-dichlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol, two nitrosoamines (morpholine and N-nitrosomorpholine) [52, 58]. The first group is formed during blanching, and the second carcinogen group is used as a corrosion inhibitor for boiler feed water. Other odor-active compounds that may be formed and released during heating of certain types of paperboard packages include acetone, 2,3-butadine, chloroform, furan, furfural, methylene chloride, carbon disulfide, and acetaldehyde. These compounds may be formed during bleaching and/or the lignin removal phases of paperboard manufacturing [51].

54.3.3 Plastic–Food Interaction

Polymers are not absolute barriers to gases such as oxygen, water vapor, aromas, and odors in packaged foods. The degree of sorption is highly dependent on types of polymer and aroma compound present in the food. Other important factors include the crystallinity of polymer, glass–rubber transition, environmental conditions, compositions of packaged food (fat content, pH, pulp content, and type of aroma compounds present) [59], free volume of polymers, sensory threshold level, intensity of aroma compounds, time and temperature of storage, diffusion rate of component in packaging and food, partition of gas phase/packaging and gas phase/food, and ratio of packaging materials to the amount of food [60].

In the case of plastics, the major source of concern is the component migration. Migration from plastics is mainly due to (i) residual components and reactants from the manufacturing process, (ii) compounds formed during conversion into packaging materials and/or packages, (iii) additives incorporated for functionality, (iv) adhesives used during conversion, and (v) time and temperature of processing and storage [24, 51, 61]. Studies show that chemical compounds in food-contact plastics migrate at a higher rate in fatty foods than in aqueous foods. The migration rate further increases with the swelling of polymers, temperature, and duration of exposure [62–66].

The toxicological implications of component migration from packages into foods are another serious problem. Thus, research on food–package interaction is growing [51]. Most plastics contain unreacted residual monomers, oligomers, and other additives, some of which are suspected carcinogens (acrylonitrile, vinyl chloride, etc.) (Table 54.7). Several

TABLE 54.7
Migrating Components from Polymer Packaging Materials to Food

Packaging Material	Migrating Component	Food	Reference
PS	Styrene dimmers/trimmers	Instant food	Kawamura et al. [123]
PS cups	Styrene	Yogurt	Nerin et al., [124]
PS	Styrene	Water, milk, cold and hot beverages, olive oils	Tawfik and Huyghebaert, [125]
Polyester cookware	Benzene	Olive oils	Jickels et al., [126]
PVC films	DEHA	Cheese	Mercer et al., [127]
LDPE, HDPE, PP, microwave packaging	Irganox 1010 (I-1010)	Food simulant liquids (FSL)	Goydan et al., [128]
	cPET		Begley and Hollifield, [129]
PVC films	Dioctyladipate	Cheese sausages	Macias et al., [130]
PVC films	DEHA	Cheese	Vaz et al., [131]; Nerin et al., [132]
Polymeric material	Styrene	Dairy products	Baner et al., [133]
PP cups	DEHA	Dairy products	Franz et al., [134]
Polystyrene	Styrene/ethylbenzene	Dairy products	Ehret et al, [135]
PP cups	2-decanone	Cheese sauce	Hansen et al., [136]
PS (+recycled material)	Monostyrene	Dairy products	Van Renterghem and de Groof, [137]
PS+ABS+waxed paperboard	Mineral hydrocarbons	Dairy products	Castle et al., [138]
Wax coatings	Mineral hydrocarbons	Cheese, sausages	Castle et al., [139]
Polymer	Dioctylphthalate	Milk	Gortseva et al., [140]
PS	Monostyrene	Milk	O'Neil et al., [141]
PP	Monomers	Yogurt	Castle et al., [142]
PS	Styrene	Food oil	Lickly et al., [103]
PS	Styrene	Cheese, dessert, meat products	Hammarling et al., [143]
PVC	DEHA	Cheese	Page and Lacroix, [144]
LDPE	Naphthalene	Milk	Lau and Wong, [145]
ABS	Mineral hydrocarbons	Dairy products	Jickels et al., [146]
PC	Bisphenol-A (BPA)	FSL	Biles et al., [68]
PVC films	DEHA	Bread, olive oil, cheese, meat	Petersen et al., [147]
PVC	DEHA	Microwave fatty foods	Lau and Wong, [65]
PE	Nano silver	Butter, orange juice, bread, cheese	Metak et al., [19]
Ny//Coated PET//CPP	Silicon-metal oxide high barrier coating	FSL	Dhawan et al., [17]

monomers have been linked with health problems, the most significant being vinyl chloride. The National Health and Medical Research Council standard in Australia is <50 ppb vinyl chloride for utensils, <10 ppb in film, and 0 ppb in foods [42]. Symptoms of vinyl chloride monomer poisoning are now well-documented. If it is present in the air at a greater concentration than 500 ppm, poisoning occurs [42]. The residual monomer styrene from PS cups and trays has been shown to migrate into food, including dairy products. Estimated daily styrene exposure is 18.2 to 55.2 μg for individuals, with an annual exposure of 6.7 to 20.2 mg [24]. Depending on the type of food product, the threshold values for styrene monomer vary. The styrene threshold amount is very low in water (taste threshold: 0.022–0.37 mg/kg) and air (odor threshold: 0.050 mg/kg), whereas it is 0.2 to 0.3 mg/kg for orange fruit juice drink, 1–3 mg/kg for oil-in-water emulsions of 15–30% oil, and cocoa powder (10–20% fat), and greater than 3 mg/kg for butter, cream (33% fat), and condensed milk (10% fat) [67].

Polycarbonate plastic is used in the manufacture of food storage containers, and bisphenol-A is a principal reactant in its preparation. Residual bisphenol-A in polycarbonate bottles migrates to liquid foods, and can be determined at low ppb levels [68]. Low-molecular-weight oligomers can migrate from PET trays into foods [6, 69]. The cyclic oligomers of butylene terephthalate can be used only in PET, PC, PS, rigid PVC, and PBT plastics with the maximum concentration of 1% (w/w) [5]. In general, packaging materials can alter the flavor profile of packaged foods by absorbing flavor compounds, chemically reacting with food components to produce off-flavors, and/or releasing components that produce off-flavors into food. Taint is defined as odor or off-flavor in food and is the major reason for consumers' rejection of food. Taint in food and beverages can come from contact polymers containing PS, PA, polyolefins, PVC, and epoxys, noncontact substances such as printing inks, coloring agents, antioxidants, and recycled materials [70]. Nestle's Central Packaging Laboratory found that taint in food is related to solvents used in packaging (28%), degraded PE (24%), halogenated phenols (15%), styrene (15%), degraded paper (3%), and other sources (15%) (EU/2011).

Migration in plastics packaging refers to the transfer of compounds from the plastic to the food product by leaching

or diffusion. Direct contact between plastic and a food product can result in leaching of packaging components into the product, changing the flavors of the food. The main problematic components are amides (slip agents), monomers and oligomers, heat-degradation products from the polymer base, and ink components. Migration may also occur from the food to the plastic, which can result in plasticizing of the package if the vapors are water or certain solvents. This can decrease mechanical strength. The food may lose valuable volatiles, such as odors, carbon dioxide, water, or flavors that increase with higher temperatures. For example, a fruit juice in polyethylene will lose limonene to the plastic (scalping) and increase ascorbic acid degradation [42]. A study shows that PET at 37°C absorbs limonene almost seven times more than at 5°C [71]. Limonene is a nonpolar unsaturated hydrocarbon with a high affinity for nonpolar polymers. Citrus and apple aroma compounds can be scalped by polymers [72]. Dye materials from foods may be scalped by nylon-6 polyamides, altering the color of foods [70]. More details on flavor–food packages interaction are given by Linssen and Roozen [73] and Sajilata et al. [72].

Instead of testing for each possible migratory compound, a test for the total migration of all compounds from a packaging material is usually used. The migration of small molecules in plastic under high-temperature conditions is known to pose problems [74]. The transfer of aromas or flavors leads to tainting of beverages filled for the second time. The single largest groups are monomers and oligomers with molecular weights up to 1000 [61]. The chemical nature of monomers and oligomers depends on the polymers. Vinyl chloride monomer, acrylonitrile monomer, and styrene are three monomers that have received substantial attention [51]. Their migration is very important because of potential toxic and carcinogenic effects. Monomer- and oligomer-mediated flavor problems or off-flavors are equally important. Styrene monomer has a very low sensory threshold and imparts strong odors to foods [75]. In general, high-molecular-weight (around 1000) oligomers found in polyolefins are paraffinic in nature and impart oily off-flavors to foods [61]. Polystyrene in plastic cups has been reported to produce off-flavors [76]. Coffee creamer and condensed milk packed in PS portion packs have exhibited styrene taint [67]. Changes in flavor and odor of butter packaged in waxed parchment paper during refrigerated storage have also been attributed to styrene migration [77].

Other compounds that are produced during plastics manufacturing and cause flavor problems due to migration include aldehydes, ketones, carboxylic acids, hydroxyl acids, catalyst residues, and solvents [51]. Solvents used during polymerization reactions are present at very low levels in the raw resins, but some foods are sensitive to them at the ppb level [78]. A common catalyst 2,2'-azobisisobutyronitrile used during the polymerization of polyvinyl chloride, acrylonitrile, and polystyrene decomposes into tetramethylsuccinonitrile at around 100°C [79]. Migration of this is extremely undesirable, since it is an acute central neurotoxin [80, 81]. During extrusion of the plastic material, temperatures may reach 250°C and higher. At these temperatures, the antioxidants in the polymer are rapidly lost. Free radicals may form on the surface of the material and in food contact. These oxidizable sites or contact points may cause autocatalyzed oxidation of food products [2].

Polyolefins give a variety of thermal degradation (oxidation) products, including aldehydes, ketones, and acrolein [61]. Unsaturated carbonyl compounds present in PE can give "PE-odor" ranges from stale, stuffy, candle-like, musty, to rancid or soapy [82]. A wide range of odor descriptors are used to depict the off-flavor characteristics of foods and beverages, e.g., a "musty" odor in orange juice packed in PP materials [83], a stale and fruity flavor in milk packed in HDPE [84], and a "plastic" odor in corn chips packed in HDPE packages [85]. Compounds associated with the "plastic"-type off-odor of HDPE are usually 1-alkenes. The "plastic"-type off-odor may also relate to other chemical compounds, including styrene, benzothiazole, methylacrylate, trans-2-nonenal, and methylmethacrylate. However, plastic-like off-flavors developed in foods do not necessarily originate from packaging materials. For example, a plastic-like off-flavor in sorbic acids is generated from their degradation products [60]. Polyvinyl chloride contains allylic chlorine atoms that are readily released during exposure to minimal heat and render the material thermally unstable. Polystyrene is relatively heat-stable, but evolves about 0.2% styrene monomer during extrusion [61].

A study on the thermal decomposition of low-density polyethylene at similar conditions during extrusion revealed 44 degradation by-products, representing hydrocarbons, alcohols, aldehydes, ketones, acids, cyclic esters, and cyclic ethers [86]. The major degradation products were formic acid, formaldehyde, acetic acid, and acetaldehyde. Formaldehyde and acetaldehyde are odor-active compounds associated with strong off-odors in packaged foods [78]. The migration of acetaldehyde from PET is of primary concern with respect to migration and off-flavor development in foods [52, 60].

Adhesives are used with some multilayer packaging materials to bond dissimilar materials together and to fabricate (seal) certain types of packages. Many adhesives contain solvents that may migrate into foods [61]. Solvents are also part of ink systems used for printing packaging materials and can impart off-flavors to packaged foods [78]. However, the proper drying of printing materials may completely eliminate solvent migration from adhesives and printing inks [78]. Most solvents are volatile and highly odor-active. Off-flavor problems due to the migration of solvent residues in various products have been reported, including toluene, p-tertiary butylphenol, ethyl acetate, ethanol, isopropanol, toluene, acetone, methyl ethyl ketone, and hexane [51, 67]. For example, the relative threshold value of toluene in coffee is 100–500 and 10–20 mg/kg for odor and taste in order to receive consumers' acceptance [67]. Volatile compounds can be generated when packaged food in plastic is heated in microwave ovens [87]. Different local and international regulatory authorities provide a lower limit to migration from packaging materials below which regulations would be deemed not to apply. An interaction product between packaging and food may also impart an off-odor to food. For example, cooked ham packed in a PA/inomer has been reported to have a "cat-urine"-like odor associated with

methylmercaptopentane, which forms during food-packaging interaction [82].

54.3.4 Stickiness and Packaging

When food sticks to packaging surfaces, this can be either desirable or undesirable to both the processor and the consumer. For example, retaining the quality of sausage products is known to be closely related to the degree of adhesion of meats to the casing. On the other hand, the adhering of a food product to the contact surface can result in product loss, and in some cases, poor product appearance [88]. Adhesiveness or stickiness between the foodstuff and the packaging surface is a complex phenomenon. The word adhesion is broadly used to denote the sticking together of two materials, with or without an intermediate layer. It is an interfacial phenomenon in which the food packaging system generally involves a liquid–solid interface or solid–liquid interface. There are various theories or mechanisms of adhesion described in the literature. These include electrostatic, diffusion, mechanical, chemical, surface energetic, adsorption, and wetting [88]. Some materials and additives for polymers are designed to be effective at the surface of the material. Examples include antifogging agents, some anti-static agents, slip agents, and anti-block agents. Such materials were developed to have an inherent incompatibility with the polymer matrix. Gibb's free energy considerations lead to these types of materials accumulating at the surface, where total energy is minimized, producing the most stable configuration. There may be other cases where surface effects are not intended, but incompatibility with the matrix or other factors leads to an accumulation of the material at the surface [26].

54.4 SAFETY AND LEGISLATIVE ASPECTS

In recent years, consumer awareness about the safety and wholesomeness of foods has increased dramatically. Food contaminations and safety are a subject of intense study by many scientists. Consumer concerns pertain to food additives, both those added intentionally and those ending up in the food from, for example, the packaging material or processing equipment. The variety of substances used in food packaging is considerable. Many of these substances are potentially toxic, harmful, and can migrate into foods. In the early 1980s, numerous toxicological studies of several commonly used plasticizers demonstrated carcinogenic effects in rodents and potential estrogenic effects in humans. From a food safety point of view, it is critical to understand the toxicity of such substances at the concentrations at which they appear in the food from the packaging material.

In order to protect consumers from the migration of harmful substances from packaging to food, different countries have developed various regulations. The approaches adapted by the USA and the EC will be discussed here, since they account for the larger proportion of food packaging materials used. The FDA and the Center for Food Safety and Applied Nutrition (CFSAN) provide guidance and regulatory aspects for food. Regulatory guidelines for food-contact materials and allied aspects are documented in Parts 174 to 178 in Title 21 under the CFR [89, 90]. The regulations specify the limits of global migration of polymeric resins from packaging. The time/temperature/solvent conditions for the short-term extraction tests used to test compliance are also noted in the CFR. The likelihood of a substance posing a health hazard depends on its dietary concentration and toxic potency. The agency considered both factors in establishing a threshold of regulation level. Hence, the extent of migration of harmful substances to food that is trivial to food safety concerns is also proposed. For example, the use of BPA-based PC resins and coatings in baby food containers and infant feeding bottles is completely prohibited due to BPA migration. The FDA and CFSAN estimated new daily BPA exposures for infants and adults are 0.2 to 0.4 μg/kg body weight/day and 0.1 to 0.2 μg/kg body weight/day, respectively [24].

The Europe Commission of the European Communities (CEC) has implemented Directives for packaging materials. The first relevant CEC Directive was issued in 1976, which also proposed analytical test methods to enable limits. The EC has listed thousands of additives and monomers as potential migrants. Many are authorized with restrictions or specific migration limits, and several analytical methods have been standardized. The Directives adopted by the EU member states concerning migration can be divided into three groups: (i) Directives applicable to all materials and articles, (ii) Directives applicable to one category of materials and articles, and (iii) Directives related to individual substances [5]. In general, the Directives introduced limits upon the overall migration from plastics into food and food simulants. In addition, specific migration or composition limits for free monomers in the final article have been set for some monomers.

Currently, the limit for overall migration is set at 10 mg/dm^2 or 60 mg/kg of food simulants, assuming that a standard package has a food-contact area-to-volume ratio of 6 dm^2/L. It also includes the lists of permitted monomers, with restrictions for specific monomers. The FDA has determined that most known carcinogens pose less than a one in a million lifetime risk if present in the diet at a level of 0.5 μg/kg. In Australia and New Zealand, Food Standard Australia New Zealand (FSANZ) provides regulatory standards for food and food safety. The Australian Standard AS 2070-1999 provides guidelines for plastic materials in food-contact use, which are based on regulatory guidelines provided by the FDA and EU. In Canada, food inspection standards are monitored by the Canadian Food Inspection Agency (CFIA) and the Health Products and Food Branch (HPFB) under Health Canada, which is responsible for implementing the standards and policies.

54.5 FINAL REMARKS

The migration of package components is a time–temperature dependent phenomena. Packaged food processed and/or stored at higher temperatures for a longer duration shows higher migration. Additionally, the polarity of foods and

food-contact materials, polymer swelling, and initial concentration, molecular weight, and molecular size of components influence migration adversely. Commercial software is helpful in determining migration into food. However, systematic study and experimental work with appropriate analytical techniques are necessary to determine overall migration and migration of compounds of particular interest, depending on the toxicity level of those compounds. These factors must be considered for intelligent selection and design of food packaging for safe human consumption.

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55 Bioactive Food Packaging

Ida Idayu Muhamad and Nozieana Khairuddin

CONTENTS

55.1 INTRODUCTION

Nowadays, food security has become a major issue; hence the introduction of active and bioactive packaging, particularly antimicrobial (AM) food packaging, could play a role in food safety and security assurance. Packaging plays an important role in the whole food chain "from the field to the consumers' tables." Furthermore, food packaging has developed strongly during recent years, mainly due to the increased demands on product safety, shelf-life extension, cost efficiency, environmental issues, and consumer convenience [1, 2]. Packaging is the technology of enclosing or protecting products for distribution, storage, sale, and use. The principal function of packaging is the protection and preservation of food from external contamination. This function involves the retardation of deterioration, extension of shelf life, and maintenance of quality and safety of packaged food. Biodegradable polymers fulfill most of these functions without causing any threat to the environment. Owing to increasing urban lifestyles and global population trends, the demand for packaged, frozen, and ready-to-eat foods has witnessed a significant surge in recent times. With the supply of exotic fruits and vegetables, meat products, and frozen foods transcending geographical boundaries, the packaging industry has been focusing on the development of solutions that provide maximum food security while maintaining nutritional value at competitive prices [3].

Packaging poses new challenges to satisfy its safety requirements as compared to traditional packaging due to its deliberate interaction with the food and/or its active environment (i.e. migration of substances from packaging to food, incorrect use of the packaging due to insufficient labeling, non-efficacious operation of the packaging) [4, 5]. Food and beverage packaging has dramatically shifted from traditional to advanced packaging. Traditional packaging only addresses issues related to protection from external factors. However,

advanced packaging interacts internally (i.e. active packaging) and externally (i.e. smart or intelligent packaging) with the environment and enhances the visual appeal of the products. Therefore, foods and beverages and packaging manufacturers are looking for ways to effect physical, chemical, and microbial textures changes inside the packaged food. The requirement for active and smart or intelligent packaging is changing rapidly due to global awareness of environmental issues. The concerns are not only on the materials and production, but also on legal aspects related to environmental concerns. The focus of packaging in the past has been on the appearance, size, and integrity of the package. A greater emphasis on safety features associated with the addition of functional active agents and natural, often bio-based components used for food packaging explains the current development in bioactive packaging technology.

55.2 ACTIVE AND BIOACTIVE PACKAGING

55.2.1 Concept of Packaging

55.2.1.1 Active Packaging

Active films can be considered an evolution in food packaging since these interact with food by releasing some substance or absorbing at a constant rate over time, and improve food quality and safety. Based on the European Union Guidance to the Commission Regulation (EUGCR) No 450/2009, active packaging is a type of food packaging with an extra function, in addition to that of providing a protective barrier against external influence [6]. Surveys conducted by Brody (2001) have shown that active packaging technologies involve interactions between the food, the packaging material, and the internal gaseous atmosphere [7]. Previously, food packaging materials used to provide only barrier and protective functions. However, various kinds of active substances can be incorporated into the packaging material to improve its functionality and to give it new or extra functions. Such active packaging technologies are designed to extend the shelf life of foods while maintaining their nutritional quality and safety [8–10]. The main objective is to extend the shelf life or to improve the quality and safety of the packed food. They use antioxidants, antimicrobials, and other natural/synthetic molecules to achieve this goal. For example, when anti-microbial systems, such as silver-based compounds, are incorporated into conventional polymers such as polyethylene and polypropylene, it is called active packaging.

55.2.1.2 Bioactive Packaging

Suitable bioactive compounds incorporated into the package wall include phenolic compounds, such as phytoestrogens, carotenoids, organosulfur compounds, plant sterols, suitable dietary fiber, prebiotics, and enzymes [11, 12]. Similar to the active packaging concept, when bio-substances, such as oils, chitosan, and flavonoids, known for their antimicrobial, antithrombotic, antioxidant, anti-inflammatory, cholesterol-lowering, and anticancer properties are incorporated into packaging materials, it constitutes bioactive packaging. Bioactive packaging is expected to create a biological mechanism or interaction between its components and develop synergistic effects toward prolonging the shelf life of packaged food while simultaneously being able to communicate with the consumer on unexpected changes of the packed food leading to spoilage and quality changes. This is a novel concept in technologies intended to help in the production of functional foods, whose bioactive principles and actuators are devised within packaging or coating materials [13]. Therefore, it gives rise to a novel conceptual approach to developing functional foods, while setting the roots of a new packaging technology, in which a food package or coating is given the unique role of enhancing the food impact on the consumer's health [14]. The technologies include novel integration technologies, micro- and nano-encapsulation, and enzyme encapsulation and/or immobilization. Moreover, novel nanocomposite structures can play a role in the controlled release of active and functional compounds from the polymeric or biopolymeric structures, and available techniques can be used to characterize the release kinetics of the various compounds [15].

55.2.1.3 Active and Smart Packaging or Intelligent Packaging

Active and smart packaging systems can provide several benefits to the quality and safety of food. The active systems aim at the shelf-life extension of the food products by keeping their quality for a longer time; the smart systems aim to monitor the quality of the food product or its surrounding environment to predict or measure the shelf life better than a best-before date [16, 17]. Han et al. [18] stressed that "smartness" in packaging can have many meanings, and covers a number of functionalities, depending on the product being packaged, such as food, beverage, pharmaceutical, or household products [18]. Han et al. [18] elaborated the examples of current and future functions of "smartness" [18]: (i) retain the integrity and actively prevent food spoilage (i.e. extend shelf life), (ii) enhance product attributes (such as look, taste, flavor, aroma), (iii) respond actively to changes in the product or in the package environment, (iv) communicate product information, product history, or other conditions to the user, (v) assist with opening and indicating seal integrity, and (vi) confirm product authenticity and act to counter theft.

Smart packaging, which is also known as intelligent packaging, was invented to improve the quality and value of the product [16, 19]. There are several types of intelligent packaging technologies: (i) time temperature indicators (TTI's), (ii) radio frequency identification tags (RFID), (iii) electronic article surveillance (EAS) tags, (iv) electromagnetic identification (EMID) tags, (v) digital watermarks, and (vi) freshness indicators

55.2.2 Types of Bioactive Packaging

Types of bioactive packaging are based on the function of the incorporated bioactive agents [20]. These aspects are discussed in the following sections.

55.2.2.1 Inhibition of Microorganisms or Antimicrobial Packaging

This type prevents pathogenic spoilage/microorganism growth using antimicrobial agents. The introduction of antimicrobial agents into food packaging material could help to prolong the shelf life of food products by inhibiting the growth of microorganisms. Antimicrobial polymers can be used in several food-related applications including packaging [21–23]. They can extend the shelf life and promote safety by reducing the growth rate of specific microorganisms from direct contact of the package with the surface of solid foods (e.g. meats, cheese) or in the bulk of liquids (e.g. milk or meat exudates).

To prevent the development and spread of spoilage and pathogenic microorganisms via meat foodstuffs, antimicrobial packaging materials could be a potential alternative solution. Instead of mixing antimicrobial compounds directly with food, incorporating them in films allows the functional effect at the food surface, where the microbial growth is mostly found to be localized [10]. Antimicrobial packaging includes systems such as adding a sachet into the package, dispersing bioactive agents in the packaging, coating bioactive agents on the surface of the packaging material, or utilizing antimicrobial macromolecules with film-forming properties or edible matrices. The potential of these technologies is evaluated for the preservation of meat and meat products [10].

Additionally, antimicrobial packaging materials could also be self-sterilizing. If the packaging materials have a self-sterilizing ability because of their own antimicrobial activity, these may eliminate chemical sterilization of packages using peroxide and simplify the aseptic packaging process [24]. The self-sterilizing materials could be widely applied for clinical uses in hospitals, biological labware, biotechnology equipment, and biomedical devices, as well as food packaging. Such antimicrobial packaging materials greatly reduce the need for decontamination of processed products and simplify the treatment of materials in order to eliminate product contamination. Antimicrobial (AM) polymers might also be used to cover the surfaces of food processing equipment so that these are self-sanitized when used (e.g. filter gaskets, conveyers, gloves, garments, and other personal hygiene equipment) [25].

The effectiveness of AM film to inhibit microbial growth has been reported by many researchers [26–29]. The application of AM agent into food packaging could be effectively used not only in the form of film but also as containers and utensils [8]. AM packaging materials need to extend the lag period and reduce the growth rate of microorganisms to prolong the shelf life and maintain food safety. They have to reduce microbial growth of non-sterile foods or maintain the stability of pasteurized foods without post-contamination. The basic principle of these traditional preservation methods and AM packaging is hurdle technology [30]. Figure 55.1 illustrates the comparison between conventional packaging and AM packaging.

Figure 55.1 shows that the extra function of AM packaging relative to conventional packaging is as another hurdle in preventing the total quality degradation of packaged foods while satisfying the conventional function of moisture and oxygen barriers, as well as physical protection. The AM packaging system may not contribute to the protection function from physical damage; however, it provides tremendous protection against microorganisms, which is difficult to achieve by conventional (i.e. moisture and oxygen) barrier packaging materials.

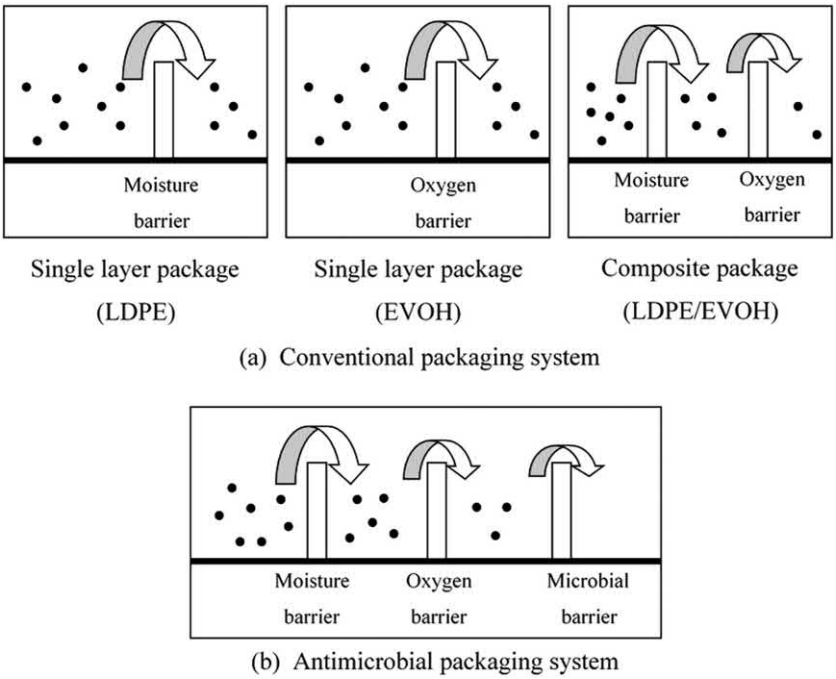

FIGURE 55.1 Hurdle technology in AM packaging system compared to the conventional packaging systems.

55.2.2.2 Inhibition of Oxidation or Antioxidant Packaging

This packaging prevents oxidative deterioration of fat components in food products responsible for off-flavor and rancidity. The addition of antioxidants blocks the oxidative chain reactions of oxygen with unsaturated fatty acids, which results in preservation. Oxygen scavenger: a. iron-based; b. metal/acid; c. metal (e.g. platinum) catalyst; d. ascorbate metallic salts; e. enzyme-based bread, cakes, cooked rice, biscuits, pizza, pasta, cheese, cured meats and fish, coffee, snack foods, dried foods, and beverages.

55.2.2.3 Gaseous Scavenging

Oxygen scavenging is necessary due to the presence of oxygen, which facilitates the growth of aerobic bacteria, yeast, and molds, discoloration, and loss of nutrient value. Ethylene scavengers used are potassium permanganate, activated carbon, and activated clays/zeolites. These are used in fruit, vegetables, and other horticultural products. The fruit crate "fruit fresh," made of corrugated cardboard, uses the hollow spaces between the corrugations to apply active materials such as kaolin clay and zeolite. Other scavenging methods include conversion of sugars, removal of cholesterol, suppressed enzymatic borrowing, juice de-bittering, nutrient etherification, aroma release, and insect repellence.

55.2.3 Sources of Bioactive Compounds

55.2.3.1 Botanical or Plant

Sources of bioactive compounds could be obtained from plant extracts, such as essential oils and phytochemicals (such as flavonoid, betel oil, cinnamon oil, clove oil, catechins, rutin, and quercetin), soy protein hydrolysate, corn protein hydrolysate, and plant-derived enzymes. Bioactive food packaging is an innovative approach for the prevention of the growth of food-spoilage microorganisms [16]. Previous researchers reported that antioxidant extracts from agro-industrial by-products exhibited antimicrobial activity and were suitable for incorporation into edible films for bioactive packaging systems. For example, a bioactive film composed of agar, green tea extract, and probiotics was applied to hake and managed to increase beneficial lactic acid bacteria but reduce the microbial growth, especially the H_2S-producing bacteria, which caused a decrease in the biochemical indexes of fish and reduced the shelf life of hake [31]. Moreira et al. (2016) reported that eucalyptus wood extract was the most active, with only 2% (v/v) necessary to inhibit *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Staphylococcus aureus*; whereas almond shell extract was less active, requiring 4% (w/v) to inhibit the growth of *Escherichia coli* and *P. aeruginosa* [32]. However, the extract from corn cobs was bactericidal against *E. coli* and *S. aureus* at a concentration of 4% (w/v). The antioxidant extracts incorporated into sodium alginate films and the maintenance of their antimicrobial properties were confirmed.

55.2.3.2 Animal

These include animal-derived peptides, enzymes, lactoferrin, hepcidin, milk protein hydrolysates, polysaccharides, or biopolymers from animal sources. Chitosan is one of the most common polysaccharides in crab shells, lobsters, shrimps, and insects. It is a β-1,4-linked polymer of 2-acetamido-2-deoxy-glucopyranose (GlcNAc) and 2-amino-2-deoxy- glucopyranose (GlcN), and is investigated as a non-toxic, biodegradable, and biocompatible material. The inhibitory activity of chitosan-based edible coatings was assessed against two food pathogens (*S. aureus* and *L. monocytogenes*) and one strain involved in food alteration (*P. aeruginosa*) on model agar medium, and these showed 100% inhibition of the development of selected Gram-positive bacteria and 77% inhibition on *Pseudomonas* growth. However, the method later proved to be effective on a real cheese food product [33].

55.2.3.3 Bacteria

Gialamas et al. (2010) developed bioactive packaging based on the incorporation of *Lactobacillus sakei* into sodium-caseinate films for the control of *L. monocytogenes* [34]. The rapid growth of *L. sakei* immobilized in the film following contact with the wet medium or the food surface, and significant inhibition of the pathogen growth was observed as compared to the controlled samples under both constant and dynamic storage temperature protocols. This indicated that biopolymer-based antimicrobial films containing cells of a protective culture can be used as an effective packaging technology for improving food safety. Soukoulis et al. [35] developed a new strategy to include probiotics in baked cereal products and reported that the sodium alginate-based surface-coated edible films effectively protected *Lactobacillus rhamnosus* GG on pan bread, and during *in vitro* digestion, whey proteins reduced probiotic loss during drying and storage [35]. Meanwhile the incorporation of lactic acid bacteria [36] as probiotics [37] in edible films successfully obtained high viability of the bacteria throughout the storage study, while their physical properties were maintained stably throughout the storage period.

55.2.3.4 Synthetic or Chemical Sources

These mainly applied to moisture absorbers, oxygen scavengers, and ethylene scavengers such as potassium permanganate, activated carbon, activated clays/zeolites, glucose oxidase and catalase, lactase, alcohol oxidase, and catalase. There are numerous bioactive agents, and these are widely used in a variety of applications in the food, pharmaceuticals, and cosmetic industries. Han [30] affirmed that in the use of antimicrobial agents in the food, pharmaceuticals, and cosmetics products, the industry must follow the guidelines and regulations of the country that they are going to use them in, for example, the FDA and/or EPA in the United States [30]. This implies that new bioactive packaging materials may be developed using only agents which are approved by the authorization agencies, for example by the FDA, or notified-to-use within the concentration limits for food safety enhancement

TABLE 55.1
Some Typical Natural and Synthetic Antimicrobial (AM) Agents Used in Food Packaging

Class of AM Agents	Examples
Antibiotics	Natamycin
Bacteriocin	Nisin, pediocins
Chelating agents	Ethylene diamine tetra acetate, purophosphate, citrates
Enzymes	Lactoperoxidase, lysozyme, lactoferrin
Essential oils	Eugenol, thymol, salicylaldehyde, cinnamic acid
Fatty acids and esters	Monolaurin
Fungicides	Benomyl, imazalil
Gas	Ethanol, Hinokithiol, $Cl0_2$
Inorganics	Sulfites, sulfur dioxide
Isothiocyanates	Allyl isothiocyanate, hypothuiocyanate
Metals	Silver, copper
Mineral acids	Phosphoric acid
Organic acids	Propionic, benzoic, sorbic, acetic, lactic, malic, succinic, tartaric
Oxygen absorber	BHT
Parabens	Methyl, propylparaben
Phenolic antioxidants	Butylated hydroxyanisole, butylated hydroxytoluene 2-terbutylhydroquinone
Proteins	Conalbumin, cathepsin
Others	Reuterin (3-hydroxypropionaldhyde), hydrogen peroxide, ozone, sulfur dioxide

or preservation [30]. There are various types of bioactive agents such as those shown in Table 55.1 which could be incorporated into the packaging systems including chemicals with antimicrobials, antioxidants, biotechnology products, AM polymers, natural AMs, and gaseous [38, 39].

55.2.4 Methodology of Preparation and Mechanism of Actions

Bioactive polymer systems may be classified as migratory bioactive polymers and non-migratory bioactive polymers according to the release and their mechanism of actions and the biodegradable polymer system [33, 40]. Moreover, bioactive agents can be incorporated through immobilization depending on the mechanism of action of the agent.

55.2.4.1 Non-Migratory Bioactive Polymer System (NMBPS)

Polymer bioactivity is devoid of active components migrating from the polymer to the substrate. NMBPS is used as a moisture absorber, oxygen scavenging system, and ethylene scavenger, and it is under investigation in the area of in-package enzymatic processing and non-migratory antimicrobial packaging. NMBPS can generally be divided into two main groups [40].

Inherently bioactive polymers (e.g. polymers containing free amines) have antibacterial activity. Polymers that belong to this group are naturally bioactive themselves without any additional compound. At present, various polymers display inherent antimicrobial properties such as chitosan and UV-treated polyamide. Chitosan is a polysaccharide based on chitin and is widespread in nature, and more effective against spoilage yeast and some Gram-negative bacteria including *E. coli* and *P. aeruginosa*. Chitosan acts through binding to the cytoplasmic membrane surface, and it is possible that the outer membrane protects the Gram-negative cells. Moreover, epifluorescence microscopic results showed a possible chitosan action during a short time duration on the synthesis of nucleic acids and especially on the relative proportion of RNA compared with DNA [33]. This impact was followed by an adaptive mechanism of the cells. Polymers with immobilized bioactive compounds (i.e. polymer modified with bioactive agents) hold specific properties.

55.2.4.2 Migratory Bioactive Polymeric System (MBPS)

Bioactive agents can be released from the polymeric system as these are incorporated in the polymer matrix by different methods [41]. For example, direct incorporation methods and coating techniques allow the migration of bioactive agents. MBPS may be divided into volatile or non-volatile groups depending on the nature of the bioactive agent. Non-volatile MBPS or non-volatile active agents are incorporated directly into packaging material or placed between the package and the food. In the case of compounds attached to the packaging material, these transfer from the polymeric system to the food surface through diffusion.

55.2.5 Methods of Incorporation of Bioactive Substance

Anti-microbial (AM) packaging is part of bioactive packaging, which combines the food packaging materials with AM substances to control microbial surface contamination of foods. Appendini and Hotchkiss [25] characterized the forms of bioactive packaging accordingly by:

i. Incorporation of bioactive substance into a sachet in the package
ii. Direct incorporation of bioactive substance into the package wall
iii. Coating the packaging material with a matrix that serves as a carrier of a bioactive substance
iv. Use of either inherently bioactive polymers that exhibit film-forming properties and are chemically modified or polymers that can be chemically modified to produce bioactive properties
v. Use of bioactive edible coatings directly applied into the food

Manipulating the solvents and/or polymer structures can enhance antimicrobial adsorption. Poly(ethylene-co-methacrylic acid) films treated with sodium hydroxide and swollen with acetone showed an increased absorption and

TABLE 55.2
Antimicrobials Covalently/Ionically Immobilized in Polymer Supports

Functional Support	Antimicrobials	References
	Lysozyme	Hanusova et al. [43]
Low-density polyethylene	Bacteriocin	Storia et al. [96]
Ethylene vinyl acetate (EVA) Ionomers	Surface pre-reacted glass-ionomer (S-PRG)	Nagai et al. [97]
Polystyrene (styrofoam)	Lysozyme	Wu and Daeschel [98], Moringo et al. [99]
	Silver nanoparticles	An et al. [100]
Polyvinyl alcohol	Lysozyme	Conte et al. [101] Feng et al. [102]
Polyvinyl alcohol	Lysozyme-essential oil	
Polyvinyl alcohol poly(methyl vinyl ether-alt-maleic anhydride)	Lysozyme	Najafi et al. [103]

diffusion of benzoic and sorbic acids compared to non-treated films [25]. Immobilization of AMs by ionic or covalent linkages to polymers requires the presence of functional groups on both the antimicrobial and the polymer. Table 55.2 summarizes a few examples of ionic and covalent immobilization of antimicrobials onto polymers or other materials.

There are AMs that cannot tolerate the temperatures used in polymer processing, and these are often coated onto the material after forming or are added to cast film. For example, cast edible films, have been used as carriers for AMs and applied coatings onto packaging materials and/or foods. Previous research discovered the lytic effect of adsorbed nisin on *L. monocytogenes* cells and thus further supported the hypothesis that the use of adsorbed nisin as an antimicrobial agent on food contact surfaces may indeed be feasible [42]. Other examples include adsorption of nisin on polyvinyldichloride (PVdC), [43], polylactic acid [44], hydroxypropyl methylcellulose (HPMC) [45], acrylic/vinyl acetate-ethylene [46], and nisin/EDTA/citric solutions coated onto PVC, nylon, and LLDPE films [47]. The potential reduction in AM activity due to immobilization must be considered. Changes in conformation and denaturation of protein and peptides may result in low activity per unit area. The protection of active sites during film formation and the incorporation of dendrites are a selected way to increase the activity. Conte et al. [48] suggested that the immobilized lysozyme has the potential to reduce bacterial activity of *Micrococcus lysodeikticus* [48]. Other AM enzymes that could potentially be covalently immobilized for packaging applications include lactoferrin, sulfhydril oxidase, and bite-salt stimulated lipase [25, 49, 50].

Some synthetic polymers or natural polymers are inherently AM and have been used in films and coatings. Among natural polymers, chitosan exhibits AM activity [25, 30, 51]. It has been found that the use of agricultural biopolymers that are easily biodegradable, such as chitosan, would solve the problem of waste disposal and un-degraded polymers caused by synthetic polymers [51]. In addition, chitosan-based antimicrobial films have been used to carry organic acids and spices [52]. UV or excimer laser irradiation can excite the structure of nylon and create AM activity. A subsequent study on UV-treated nylon films showed that the surface amino groups were bactericidal, and bacterial cells were adsorbed to the surface and diminished the effectiveness of the amine groups [53].

Incorporation of AM substance into polymers has been commercially applied in drug and pesticide delivery, household goods, textiles, surgical implants, and other biomedical devices [25]. The commercialized antimicrobial compounds include silver substituted zeolite, chlorine dioxide, carbon dioxide, ethanol, glucose oxidase, and allylisothiocyanate. The rationale for incorporating AMs into the packaging is to prevent surface growth in foods where a large portion of spoilage and contamination occurs [25]. Common AM chemicals for food products are preservatives such as organic acid and their salts, sulfites, nitrites, antibiotics, and alcohols [8]. These are mixed into a wax layer for natural cheese [54]. The AM mechanism/kinetics and the controlled-release profile of potassium sorbate from starch-based film into cheeses were examined and mathematically simulated by [55]. Santonicola (2017), on the other hand, reported the release of natamycin from chitosan/methylcellulose film into the same food product [56].

Biodegradable polymers are being studied as edible coatings or film materials [57]. Ramos et al. [58] reported on the AM activity of essential oils in corn zein films. Trombetta et al. (2005) concluded that the antimicrobial effect of (+) menthol, thymol, and linalyl acetate may be due, at least partially, to a perturbation of the lipid fraction of bacterial plasma membranes, resulting in alterations of membrane permeability and in leakage of intracellular materials [59]. Besides being related to physicochemical characteristics of the drugs (such as lipophilicity and water solubility), this effect appears to be dependent on the lipid composition and net surface charge of the bacterial membranes lead to the cross of the cell membranes, penetrating the interior of the cell and interacting with intracellular sites critical for antibacterial activity [59].

A previous study showed that thymol has successfully inhibited the growth of various microorganisms. Ramos et al. [60] found the decrease of viable counts of *S. aureus* on nutrient agar upon the addition of either the carvacrol of thyme or

its constituent thymol. Klarić et al. [61] in their study of antifungal activity of thyme essential oil and thymol against molds reported that essential oil of thyme, which is rich in thymol and other antifungal components, could be used for the disinfection of moldy walls in the dwelling in low concentrations. Corbo et al. [62] suggested the use of thymol as an ingredient for producing fish hamburgers. Their study revealed that thymol in combination with modified atmosphere packaging showed the greatest inhibitory activity against *Shewanella putrefaciens* and *Photobacterium phosphoreum* [62].

A study of antibacterial activities of nisin, thymol, and eugenol against four strains of common spoilage bacteria named *E. coli*, *S. auereus*, *Bacillus cereus*, and *L. monocytogenes* concluded that thymol appeared to be the most preferable agent for research in active packaging in terms of its antimicrobial benefit [63, 64]. An ordinary feature of plant volatiles is their hydrophobic nature, and studies addressing the mode of antimicrobial action of such compounds commonly point to the cell membrane as primary target [65, 66]. Bio-actives directly incorporated into packaging polymer matrices need plasticizers and additives. These are small molecular weight (M_w) compounds, which can interfere with protein chain-to-chain hydrogen bonding, and extend, dilute, and soften the structure, increasing chain mobility, e.g. glycerol, propylene glycol (PG), polyethylene glycol (PEG), sorbitol, and water; they are generally used to improve mechanical properties of protein films [67–69]. Plasticizers are generally added into film-forming solutions to prevent film brittleness or cracking caused by intermolecular forces [67]. These illustrate how bioactives are combined with packaging to suppress microbes and improve the quality of packaged food products.

55.2.5.1 Micro- and Nano-Emulsion

Bioactives, in particular natural pigments or natural dyes with multifunctional properties such as anthocyanins, betalaine, catechin, and chlorophyll, can be prepared with microencapsulation or nanoencapsulation techniques and incorporated into biopolymer films to provide the desired functional properties of a pH indicator, antimicrobial effect, and antioxidant benefits [70–72]. In a smart packaging system, color indicators could provide information about product quality by surrounding conditions and headspace gases of packages; it can also be attached to the package surface or integrated into packages that are improved for determining metabolite residue formed during storage [73]. Temperature, microbial spoilage, package integrity, physical shock, and freshness of the packaged product can be controlled. Anthocyanins are secondary metabolites widely distributed in fruits and vegetables (e.g. red cabbage, sweet potato, bean husk, grapes), making them a promising source of natural indicators that exhibited greater color spectrum as a function of pH and also antioxidant [14, 17, 74, 75]. Several studies investigated the use of biopolymers and anthocyanins for the production of pH indicators. However, studies related to the evaluation of time, temperature, and light effects on the stability of pH indicators, especially those developed with natural colorant, need to be further detailed.

55.2.5.2 Bioactive/Enzymes Immobilization Technology

In the encapsulation or immobilization techniques, tiny substances are encapsulated in food at nanoscale range having diameters of 3 to 800 micrometers. In such a context, their efficacy is guaranteed due to their significantly improved stability and/or release of the bioactive compounds incorporated [76, 77]. This is based on their interactions with the polymeric material and on the environmental conditions of their applications such as temperature, relative humidity, and so on. The significant potential of using nanotechnology tools to improve the performance has made polymers and biopolymers the material of choice as carriers of the bioactive compounds for those types of applications [13, 78, 79].

55.2.5.3 Controlled Release of Bioactive Compounds

In controlled release technology, a migratory bioactive system can be designed as a controlled release system that plays an important role in sustaining a constant concentration of bioactive agents in food and pharmaceutical products without waste of the bioactive agent for a long period. Therefore, the migration rate of active agent from the packaging must be controlled specifically [80–82]. If the migration rate of antimicrobial agent is faster than the growth of microorganisms, the incorporated antimicrobial agent can be weakened to less than the effective critical concentration before the expected period. Several types of biopolymers including kappa-carrageenan, polylactic acid, polyglycolides, and polyorthoesters have been important in controlled release.

55.2.6 Controlled Release System in Bioactive Packaging

In food packaging, migration is used to describe the transfer of a substance from the packaging materials into a food, and it is essential in order to effectively inhibit the growth of microorganisms on the surface of food products. There has been great interest in developing AM packaging materials, which slowly release AM agents to the surface of food and inhibit microbial contamination on the food surface during food storage [12, 83, 84]. Extensive research has been conducted to develop strategies in association with controlled release. Mathematical models play a vital role in accommodating polymeric network design by identifying active compound release mechanisms and key parameters.

Most food packaging systems consist of packaging material, food, and the headspace in the package. Diffusion between the packaging material and the food and portioning at the interface are the main migration phenomena involved in this system. AM agents may be incorporated into the packaging materials initially and released into the food through diffusion and partitioning. Han's description of the migration phenomena is shown in Figure 55.2 [8].

Control of the release rates and migration amounts of the AM substance from food packaging is very important. In principle, the extractability of a compound from a plastic by a foodstuff can be determined by placing the plastic (of known

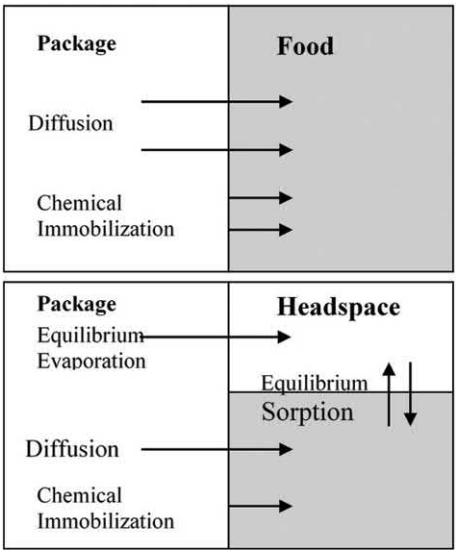

FIGURE 55.2 Food packaging systems and migration phenomena. (Adapted from Han [8].)

surface area) in contact with the food under defined conditions of temperature and time [68]. In practice, the test faces difficulties for the following reasons:

(i) The compounds present in the plastic under test may be unknown and may have been degraded during processing.
(ii) In many instances, such compounds are difficult to determine analytically in a matrix as complex as food, particularly where only small amounts are present in the extract.
(iii) Compounds other than the one of interest may also be extracted and subsequently interfere with the analytical determination.
(iv) Most of the foodstuffs are stable for short periods of time only, whereas extractability data may be required from long-term studies.
(v) Appropriate test conditions are not easy to define as a result of the wide variation in possible contact conditions likely to be encountered in practice in warehouses, supermarkets, corner shops, and household larders.

While it is always desirable to use foods themselves for extractability testing, in practice, it is seldom possible, and so food simulants have to be used instead [68]. The extractability of a compound from plastic can be determined by placing the plastic (of known surface area) in contact with the food or food simulant under defined conditions of temperature, time, and static/dynamic mode [68, 79].

55.3 HISTORY AND DEVELOPMENT

55.3.1 History and Development of Bioactive Packaging

The global nano-enabled packaging market size was USD 23.73 billion in 2015 and is expected to witness significant growth over the forecast period as shown in Figure 55.3 [85]. The key drivers impacting the demand growth include favorable food safety regulations and increasing demand for effective packaging solutions in the food and beverage and pharmaceutical sectors. Nanotechnology is one of the most rapidly growing industries, which is contributing to the growth of several industries; one such industry is the active packaging industry. Increasing usage of nano-enabled packaging in the bakery, meat, fruit and vegetable, and other processed food products is anticipated to boost demand from 2016 to 2024.

The most widespread use of active agent in the industry is synthetic chemical substances, which include alcohol, antibiotics, fungicides, and organic acid. Organic acids and their mixtures possess strong AM activity and have been used as food preservatives, food-contact substances, and food-contact material sanitizers [30]. The use of antibiotics as package additives is not approved for the purpose of AM functions and is also controversial due to the development of resistant microorganisms. However, antibiotics may be incorporated for short-term use in medical devices and other non-food products [30, 38]. Chlorine oxide, allyl isothiocyanates, and ozone are examples of the gaseous AM agents that have been successfully incorporated into packages, which offer protection in the headspace of food packaging. Ethanol vapor generation consists of ethanol absorbed or encapsulated in carrier materials and enclosed in polymer packets. The ethanol permeates the selective barrier and is released into the headspace within the package [25]. Various AM agents could be incorporated into the packaging

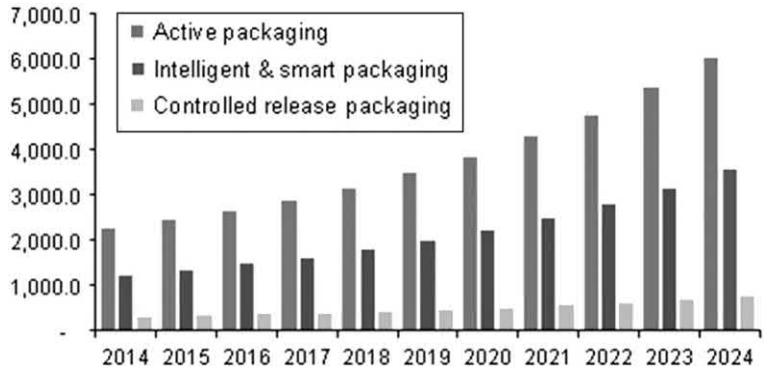

FIGURE 55.3 U.S. nano-enabled packaging market revenue by application, 2014–2024 (in USD million).

systems including chemical AMs, antioxidants, biotechnology products, AM polymers, natural AMs, and gas [16, 38, 39, 86].

Therefore, research on bioactive packaging in general is also, more widely, research on natural compounds as bioactive agents. Pandey and Kumar [87] and Abdallah [88] elaborated on the plant products that can be used as antimicrobial agents, and their review describes the current state of plant antimicrobial compounds worldwide. It studies extracts commonly in use, their structure, mode of action, stability, possible technologies by which they can be delivered, and substances being explored and tested by researchers and clinics back in 2011. Plant essential oils are a potentially valuable source of AM agents that have been shown to possess distinct AM activities against many pathogenic microorganisms [64, 89–92]. For example, the AM effects of essential oils and their active constituents against pathogenic microorganisms including *E. coli* [64, 90–93], *S. aureus* [64, 90, 91, 93], *B. cereus* [64, 91], *E. coli* O157:H7 [94], *Penicillium roqueforti*, *Endomyces fibuliger*, *Penicillium corylophilum*, *Aspergillus flavus* and *Eurotium repens* [95], *Staphylococcus epidermidiis*, *Bacillus subtilis*, *Bacillus* spp., *Micrococcus luteus*, *P. aeruginosa*, and *Klebsiella* spp. [91] have been studied.

55.3.2 Law and Regulations

Active and intelligent packaging is based on a deliberate interaction of the packaging with the food and/or its direct environment to improve food quality and safety. Such technology includes advances in delayed oxidation and controlled respiration rate, microbial growth, and moisture migration. Other examples are carbon dioxide absorbers/emitters, odor absorbers, ethylene removers, and aroma emitters, while intelligent packaging includes time–temperature indicators, ripeness indicators, biosensors, and radio frequency identification. An amendment to the EU Framework Directive for food-contact materials 89/109/EEC has led to the adoption of a new Framework Regulation (1935/2004/EC). The new Framework Regulation for Food Contact Materials (1935/2004/ EC, which was published on 27 October 2004 (5), authorizes the use of active and intelligent packaging, provided the packaging can be shown to enhance the safety, quality, and shelf life of the packaged foods. Based on the definitions laid down in Regulation (EC) No 1935/2004 and in Regulation (EC) No 450 /2009, "active materials and articles" means materials and articles that are intended to extend the shelf life or to maintain or improve the condition of packaged food. These are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food. Nevertheless, due to its deliberate interaction with the food and/or its environment, the migration of substances could represent a food safety concern.

55.3.3 Industrial Applications and Benefits

Factors to be considered in the manufacturing of active/bioactive films are (i) the chemical nature of the film, (ii) process conditions and residual antimicrobial/antioxidant activity, (iii) characteristics of antimicrobial/antioxidant substance and specific foods, (iv) chemical interaction of additives with film matrix, (v) mass transfer coefficients, (vi) physical properties of packaging material, and (vii) environmental conditions such as storage, temperature, and RH. These could be the industrial benefits of enclosing bioactives within packaging or coating materials.

55.4 CONCLUSIONS AND FUTURE DIRECTION

The purposes of packaging technology are to maintain sensory quality and extend the shelf life of food whilst at the same time maintaining nutritional quality and ensuring microbial safety. Bioactive packaging is an emerging trend and exciting area of food packaging technology with improved quality and safety resulting in innovations in packaging techniques that can confer many preservation benefits on a wide range of food products. The new advances have mostly focused on delaying oxidation and controlling moisture migration, microbial growth, respiration rates, and volatile flavors and aromas. Bioactive packaging is thus a novel set of technologies designed to give response to a number of issues related to the feasibility, stability, and bioactivity of functional ingredients for the food industries. These technologies aim to integrate bioactives within new packaging and coating material concepts and can greatly benefit from previous developments in the pharmaceutical and biomedical sectors and from the unique properties of synthetic and biomass-derived biopolymers. Research and development in response to consumer preferences gave rise to active, smart, and bioactive food packaging techniques that are purely innovative. These innovative packaging technologies contributed toward the improvement of food quality, safety, feasibility, and bioactivity of functional components. The applicability of novel and innovative packaging techniques is growing widely because of their health impact, thus resulting in reduced consumer complaints. In the near future, conventional packaging could be completely replaced by innovative food packaging techniques as these techniques are rapidly making their way into the global market.

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56 Hygienic Design and Sanitation

Mohammad Shafiur Rahman, Md. Jiaur Rahman, and Nasser Al-Habsi

CONTENTS

56.1 HYGIENIC DESIGN

The food industry is a diversified and large sector that produces, processes, and supplies much of the foods consumed by the world population. However, proper hazard management, food safety, and hygiene practices are crucial in food production and handling in protecting public health. Hygiene is a key discipline in the area of food safety and public health management. Lack of adequate hygienic practices at all stages of harvesting, post-harvesting, processing, and storage drastically increase the risk of contamination with food poisoning and food infection [1]. Therefore, hygienic design is essential to ensure safe goods supply for consumers. Sanitation is an applied science that states the creation and maintenance of hygienic and healthful conditions in an industry. Its applications refer to hygienic practices designed to maintain a clean and wholesome environment for food production, preparation, and storage [2]. This includes not only cleanliness in foods, but also improved food processing environments that prevent chemical, physical, and biological hazards from causing illnesses and injuries to consumers. One of the keys to operating a clean and sanitary facility is the design of the building and equipment. It is important to do it right the first time, as it is always much harder and more expensive to retrofit or rebuild [3].

56.1.1 Factory Facility Design

A new and renovated food factory facility should be well planned and emphasized with effective functionality, hygienic design, and construction principles to ensure sanitary operations. A hygienically designed facility can boost the healthfulness of all foods and facilitate a sanitation program effectively ([4]. Therefore, it is important to consider hygienic factors when designing food processing factories and equipment in order to ensure food safety and quality.

56.1.1.1 Factory Site

Factory site selection is pivotal in the development of hygienic operations. The design, construction, and maintenance of the factory site and buildings need special considerations from a hygienic point of view. To maintain a good standard of hygiene, a well-planned and adequate waste disposal system is essential [5]. Attention to the design, construction, and maintenance of the site surrounding the factory provides an opportunity to set up the first (outer) of a series of barriers to protect production operations from contamination. These barriers start at the site perimeter boundary, and moving inward, ending with the walls of high-care and low-care regions [6]. Thorpe [6] reviewed the number of steps that can be taken for a factory site. The important ones are as follows:

i. A food factory should not be built up near chemical plants that emit toxic odors, or near salvage yard or water disposal operations. Foodstuffs that are high in fat may pick up bad odors and flavors spontaneously during production, preparation, and processing. In addition, foodborne pathogens can contaminate the

manufactured products by air blown across them, unless special filters are added to the intake air systems [4].

ii. Use of two lines of rodent baits located every 15–21 mm along the perimeter boundary fencing and at the foundation walls of the factory, together with a few mouse traps near building entrances [7].
iii. Keeping areas immediately adjacent to buildings grass-free and covered with a deep layer of gravel or stones, which helps weed control and assists inspection of bait boxes and traps [8, 9].
iv. Making the factory site unattractive by denying birds food and harborage. This means clearing up any spillages of raw materials, avoiding keeping waste material in uncovered containers, and avoiding big trees around the site [10].
v. Premises should be purpose-built to a sanitary design with modern, easy-to-clean materials and sited in surroundings that are free from potential harborages for rodents, birds, and insects [5].
vi. Preventing the unauthorized opening of doors and windows, and using protective screens against flying insects.
vii. Use of high-pressure sodium lights in preference to mercury vapor lamps for lighting of warehouses and outdoor security systems to avoid attracting flying insects at night [7, 10].
viii. Use of good landscaping, which can reduce the amount of dust blown into the factory. Entrances into the plant and areas surrounding it should be well paved to reduce the amount of mud and dirt brought in by workers and clients [1].
ix. Use of orientating buildings so that prevailing winds do not blow directly into manufacturing areas. Doors and windows of the plant should be screened tightly to prevent the entrance of flies and other insects [1].
x. Use of proper layout of vehicular routes around the factory site to avoid soil blown into buildings [6].
xi. Plant premises and areas surrounding it should be kept completely free of all decomposing materials and pools of stagnant water that generate foul odors and are ideal breeding grounds of flies and other insects [1].
xii. Avoid the presence of flowering plants to prevent attracting flies and other insects.
xiii. The air circulated within the factory needs to be controlled, since pests, insects, rodents, and birds carry pathogens.

56.1.1.2 Floors

The floor design of a food facility is an important factor in hygienic design and sanitation. The selection of floor surface depends on the plant and its operations such as raw material collections, food production, preparation, processing, and food materials from the process falling onto the floor. The most critical hygienic issues in a food facility design are the junctions among different construction components, especially between floor finishes and stainless-steel drainage components. For designing of floors, alongside flooring materials, some important aspects such as gullies, channels, junctions, joints, and falls should be carefully considered. Junctions and joints are prone to microbial contamination. The fewer the joints, the less the hygienic risk. Intensifying the hygiene-enhancing qualities of specialist safety flooring is an important aim in safety flooring manufacture. The main characteristics of an ideal floor required are as follows:

i. The floor must be strong, fast, and abrasion-resistant.
ii. The floor must be impervious to water, nonabsorbent, and corrosion-resistant.
iii. The floor should be free of cracks, pits, and crevices.
iv. The floor must be resistant to oil, grease, chemicals, and skid-resistant
v. The floor must be a high-density type. A less porous material reduces moisture absorption and reduces microbial growth.
vi. Resistant to cleaning and sanitizing chemicals.
vii. The floor must be durable, easily maintained, and wear-resistant.

A bacteriostat in the formulation is integral to the flooring and gives the exposed surface strong antibacterial activity against gram-positive and gram-negative microorganisms [11]. A slip-resistant floor is another safety aspect in the prevention of injury-causing slips, trips, and falls. As the problem of floor surface-slip performance is relatively complex, there are many other factors involved than simply the flooring material. Thus, there is a broad range of polymeric shoe-sole materials used today as well as flooring materials. Today a wide variety of flooring solutions are available. These include safety floors studded or formulated with hard mineral additives (fine grain chemicals, for example, silicon carbide) that provide hard coefficient of friction (for strong grip) under wet and contaminated, for example, greasy, conditions [11]. Studded tiles are not recommended because of the greater difficulty of cleaning such surfaces. Ideally, surfaces that offer the greatest ease in cleaning should be used. However, the final choice should reflect a balance between ease of cleaning, slip resistance, and other factors. Joints should be grouted in proper ways. Cementitious grouts are not considered suitable hygienic applications. Epoxy, polyester, and furan resins can be used. Epoxy resins have limited resistance to very high concentrations of sodium hypochlorite and soften at temperatures above 80°C. Polyester and furan resins are more resistant to chemical attack. Floor finishes with either tiles or a synthetic resin should be considered. The choice of flooring surfaces can be broadly grouped into three categories: (i) concretes, (ii) fully vitrified ceramic tiles, and (iii) seamless resin screeds. Concrete flooring, including the high-strength granolite finishes, may be suitable in different parts of the factory. However, concrete is not recommended for high-care production areas due to its ability to absorb water and nutrients. This allows microbial growth below the surface, where it is extremely difficult to apply effective sanitation cleaning [6]. Pressed or extruded ceramic tiles are still extensively used in processing areas. In recent years, these have

been partially replaced by various seamless resin floors due to cost and wide availability. The waterproof membrane of floors should be extended up walls to a height above the normal spillage level [6].

All floors in the factory should be regularly cleaned and must be properly constructed with good drainage. Water should not be allowed to remain stagnant on the factory floor or drain because this breeds unwanted insects and flies [1]. A correct slope on the floor is also important [5]. Drainage should be accounted for in the proposed layout of equipment. Ideally, the layout and siting of production equipment should be finalized before the floor is designed to ensure that discharges can be fed directly into drains. In reality, this may not be possible for the food industry since the layout of lines may be frequently changed. Drainage should be in the middle of the factory and equipment should be placed on both sides of the drain. Equipment should not be located directly over drainage channels as this may restrict access for cleaning [6].

56.1.1.3 Walls

The wall of a food facility should be installed and maintained to ensure all food safety issues are met from the floor to the ceiling. A number of technical factors such as hygienic characteristics, insulation properties, and structural characteristics need to be taken into consideration for walls. A U-shaped channel between the floor and wall junction should be used for easily cleanable and watertight junction. The walls must be constructed of impervious, nonabsorbent, washable, nontoxic materials, and have smooth crack-free surfaces [6]. Schmidt and Erickson [12] suggested some important characteristics of an interior wall of a food facility in hygienic design and sanitation. A cleanable and sanitary ideal wall for a food facility is one that should have the following criteria:

i. The wall should be flat, smooth, and made of concrete/insulated metal panels (IMPs).
ii. The wall must be a high-density type. Less porous material reduces moisture absorption and reduces microbial growth.
iii. The wall must be free of pits, bangs, checks, and crevices.
iv. Junctions between walls and ceilings should be rounded.
v. Joints between walls and floors should be covered.
vi. Wall should be impervious, nonabsorbent, and corrosion-resistant.
vii. The wall should be resistant to cleaning and sanitizing chemicals.
viii. The wall should be durable, easily maintained, and wear-resistant.
ix. The wall must be precisely installed, stuck down, and covered.

56.1.1.4 Doors, Windows, and Ceiling

Doors, windows, and ceiling also need to be properly designed from the point of sanitary operations. Doors allow direct entry of pests and flies that may spread foodborne pathogens in the working area of a food plant. A double-access door installed with automatic opening and closing could reduce pests and fly contamination. Although windows are essential for an effective environment and adequate lighting within a food plant, they may pose a sanitation hazard because of their breakage and contamination from pests, dust, and other sources [4]. However, if it is essential to install windows in a factory facility design, it is best if they are made of unbreakable polycarbonate material [13]. Widows must have nets to prevent insect entering into the production areas. The ceiling of a factory facility must be a smooth concrete slab of exposed double tees with caulked joints. It should be enclosed in concrete, granite, or the equivalent to avoid overhead areas that collect dust and debris, or provide rodent runways or insect harborage [4].

56.1.1.5 Lighting

Lighting in all areas of the plant should be sufficient for the adequate performance of all assigned duties. Areas where cleaning, sanitizing, sorting, picking, and product inspection are done should be provided with sufficient nonglazing light to enhance work performance [1]. Lighting should be used in such a way to avoid attraction of insects. Electro-blades could also be used to trap and kill insects and flies. Lighting stuffs, for example, LED light bulbs should be equipped with break-resistant lenses or shatterproof shielding and designed to be moisture-resistant and cleanable.

56.1.2 Equipment Design

Good hygienic design of equipment, which should be installed in such a manner as to minimize the chances of contamination occurring and to facilitate housekeeping and sanitation programs [6]. All equipment must be designed and constructed so that all internal contact points and external surfaces may be cleaned. Each piece of equipment has its own peculiar areas where microorganisms might proliferate, and hazard analysis of any weak points should ensure their removal, and indicate control and monitoring [5].

Legislation on the hygienic design of food-processing equipment or the hygienic maintenance of this equipment is rather vague [14]. In Europe, the most important legislation giving criteria for hygienic design of equipment is the Council Directive on the approximation of the laws of member states relating to machinery (89/392/EEC, revised 98/37/EC), which contains safety requirements and the basic principles of hygienic design [15]. National standards and/or directives applicable to the hygienic design of food machinery are available, but only a few international standards exist, directed mainly at the dairy industry [16]. A basic standard about hygiene requirements for the design of machinery is ISO 14159:2002 (ISO, 2002). The European Committee for Standardization (CEN) issues standards for equipment manufacturers to be able to fulfill the requirements of the directive. One important basic standard is Standard EN 1672-2 "Food-processing machinery—Safety and hygiene requirements—Basic Concepts—Part 2; Hygiene requirements" [17]. However, there are also guidelines and methods published,

e.g., by the European Hygienic Engineering and Design Group (EHEDG, www.ehedg.org), 3-A Sanitary Standards Inc. (3-A, www.3-a.org), and NSF International (www.nsf.org) available for helping in the design of new hygienic equipment. According to the EHEDG guidelines, constructions that cause problems include dead ends, sharp corners, low-quality seals, and joints [18, 19].

Equipment can be classified into three groups:

i. Aseptic equipment (high demand): Equipment that can be cleaned in place (CIP) and freed from microorganisms and spores (sterilization in place, SIP) without any dismantling.
ii. Hygienic equipment class I: Equipment that can be cleaned in place and freed from relevant microorganisms without dismantling.
iii. Hygienic equipment class II: Equipment that can be cleaned after dismantling and that can be freed from relevant microorganisms by sterilization, pasteurization, or chemical treatment after reassembling [20, 21]. It needs greater care in the location of adjoining equipment to prevent inefficient discharge of product from one unit to the next.

Valves are essential components of all food processing plants, and the quality of the valves used strongly influences the microbiological safety of the food production process. Valves for food contact use must therefore comply with strict hygienic requirements. It is important to keep the following conditions in mind: (i) pits and crevices must always be avoided; (ii) sharp edges should be avoided; (iii) screw threads should not be used; and (iv) dead ends, which may trap product or prevent adequate cleaning, should be avoided. Valves must be as short as possible and must be installed in a drainable and cleanable position if dead ends are unavoidable, and there should be as few seals as possible in a valve. During processing, the compressibility of the sealing should not be exceeded. More details are given in the European Hygienic Equipment Design Group Report [20]. In Europe, the valve design must meet the criteria as shown in Table 56.1 [22].

The guidelines for basic design and safe use of double-seat mix-proof valves in food processing, along with an outline of its benefits and applications are provided by EHEDG [23]. This type of valve prevents intermixing of ingredients or cleaning fluids during normal use. Other EHEDG [23] updates provide general hygienic design criteria for the safe processing of dry particulate materials. Figure 56.1A shows the possible design faults for a liquid holding tank and a better design is shown in Figure 56.1B. The design criteria for handling dry materials must consider (i) the eventuality of disassembly/accessibility for cleaning and inspection, (ii) the moisture content of the product, and (iii) safety aspects, including the formation of dust and exposure to it.

Mechanical seals are commonly used for pumps, agitators, mixers, and other types of rotary equipment. Dynamic sealing takes place at the interface between a stationary seal ring and a rotating ring. It is accomplished by using perfectly flat face surfaces and using the product pressure along with spring force to press the components together. Inappropriate design of mechanical seals can cause contamination of a food product by microorganisms and/or residues, which may be toxic or allergenic. The EHEDG [21] update presents the details of the design of mechanical seals for hygienic and aseptic applications. The general design criteria are presented as

i. The parts of the seal in contact with product must be suitable for CIP and SIP according to EHEDG guidelines.
ii. The nonproduct side of the seal shall be cleanable and capable of being disinfected and shall not contaminate or have any adverse influence on the food.
iii. Product-contact surfaces have to be smooth and crevice-free.
iv. The nonproduct side must be as smooth and as crevice-free as possible.
v. Dead ends are not permitted on the product side.
vi. Springs are not permitted on the product side.
vii. Designed corners must have a minimum radius of 3 mm.

Materials used for components in mechanical seals must (i) be nontoxic; (ii) be anticorrosive; (iii) be crevice-free; (iv) be

TABLE 56.1
Valve Design Standards in Europe

1. Council Directive 98/37/EC
2. CEN EN 1672-2 requirements
3. ISO 14159 hygiene requirements for the design of machinery
4. Requirements set out in the EHEDG guidelines
- Doc. 8: Hygienic equipment design criteria, 1993
- Doc. 9: Welding stainless steel to meet hygienic requirement, 1993
- Doc. 10: Hygienic design of closed equipment for the processing of liquid food, 1993
- Doc. 13: Hygienic design of equipment for processing, 1996
- Doc. 14: Hygienic design of valves for food processing, 1996
- Doc. 16: Hygienic pipe couplings, 1997

Source: EHEDG Update [23].

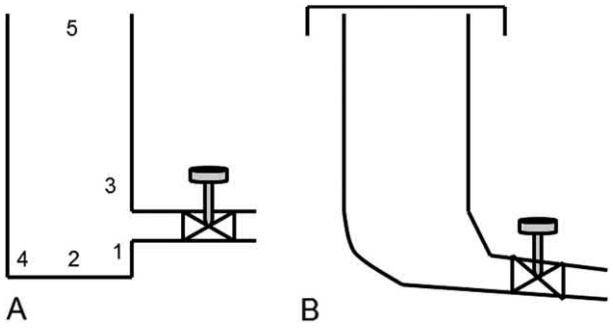

FIGURE 56.1 Design of a liquid holding tank. (A) Poor design (1: dead end, 2: no slope on the bottom, 3: sharp exit, 4: sharp corner, 5: no cover). (B) Hygienically improved design.

nonabsorbent; (v) not transfer undesirable odors, colors, or taints to the product; (vi) not contribute to the contamination of food nor have any adverse effects on the food; and (vii) have surfaces and coatings that are durable, cleanable, and capable of being disinfected; without breaks, and resistant to cracking, chipping, flaking, and abrasion; and prevent penetration of unwanted matter.

All equipment must be rinsed and sanitized on a daily basis and whenever there is a change in shift, if this is appropriate [1]. All surfaces in contact with food and outside surfaces of equipment must be cleanable. Surface roughness has a significant influence on cleaning ability. A greater surface roughness requires longer cleaning time [20]. Geometry appeared to be one of the main factors in hygiene, emphasized by the way the equipment is connected to the CIP circuit [24, 25].

56.2 GOOD HYGIENIC PRACTICE

Practicing good hygiene is an important part of hygienic design and sanitation in a food facility. Good hygiene is essential to ensure the products manufactured by a food plant and sold are safe to consume and free from contamination. Poor worker and equipment hygiene is the potential contributing factor in the contamination of the products causing foodborne illness outbreaks.

To ensure food industry staff members conform to personal hygiene requirements, two issues must be considered: (i) the environment within which the staff operates and (ii) the quality of the staff members. From a food hygiene point of view, the quality of the working environment depends on the facilities or equipment provided, which include toilets and protective clothing. The quality of staff depends upon their health, hygiene, habits, attitude, beliefs, and education or knowledge level on hygiene [26].

56.2.1 Purpose of Sanitation

Sanitation is important from legal, economic, quality, and food safety standpoints. Sanitation programs should be integrated with a company's hazard analysis and critical control points (HACCP) or food safety programs [3]. Giese [3] mentioned that good sanitation is much more than just good common sense; sanitation also entails getting the message across to all plant personnel and not just those involved with handling food. Sanitation consists of two parts: (i) cleaning and (ii) sanitizing. Cleaning means the removal of the residue of food, dirt, dust, foreign material, or other soiling ingredients or materials. Sanitizing means the effective bactericidal treatment of clean surfaces of equipment and utensils [1, 27]. Ideally, disinfectants should have the widest possible spectrum of activity against microorganisms (viruses, bacteria, fungi, and spores) in a time relevant to application contact times [28].

The purpose of sanitation are that it (i) increases the chance of complying with regulatory requirements, (ii) can prevent a catastrophe, (iii) enhances or facilitates an effective quality assurance program by increasing the acceptability and storage life of food by suppressing microbial population, and (iv) can save energy and retard the spreading of flora throughout the establishment [29]. There is an increasing trend in food production toward products with a short shelf life with low severity of processing. This demands higher standards of hygiene [30].

Cleaning and sanitizing are also important elements of any sanitation program. Good cleaning compounds must be economical, nontoxic, noncorrosive, noncaking, nondusting, easy to measure or meter, stable during storage, and easily and completely dissolved. The selection of these compounds depends on the area and equipment to be cleaned, soil types, and their attachment characteristics [29]. Different types of cleaners and sanitizers are available. There is value in working with suppliers not only to select the best materials to meet industry needs, but also to use this resource to ensure that employees have been properly trained in their use [3]. Surface-attached organisms were generally found to be less susceptible to disinfectants than suspended organisms. In practice, however, many factors are present in the factory environment that may affect the resistance of bacteria prior to disinfection [28]. The cleaning compounds are classified by Marriott [29] as alkaline cleaning compounds (strongly alkaline, heavy-duty alkaline, and mild alkaline cleaners), acid cleaning compounds (strongly acid cleaners, mildly acid cleaners, and solvent cleaners), synthetic detergents, alkaline soaps, phosphate substitutes for laundry detergents, solvent cleaners. More details about cleaning compounds and sanitizers are given by Marriott [29].

56.2.2 Strategy for Establishment of Sanitary Practices

A planned sanitation maintenance program is essential to meet legal requirements; to protect brand and product reputation; and to ensure product safety, quality, and freedom from contamination. All phases of food production and plant sanitation should be included in the program to supplement the cleaning and sanitizing procedures for equipment and factory facilities [29].

56.2.2.1 Reduction of Food Contamination Sources

Foods not handled in a sanitary way may become contaminated from processing equipment; employees; soil, air, and water; sewage; and insects and rodents. Contamination can be reduced through effective housekeeping and sanitation, protection of food during storage, proper disposal of garbage and litter, and protection against contact with toxic substances [29].

56.2.2.2 Personal Hygiene and Food Handling

Hygiene can be effectively controlled in the food industry through good manufacturing practice (GMP) and involves everyone. Good personal hygiene practices of personnel working in or visiting the production area are important. Additional requirements apply to personnel working in high-care areas. Personnel in the production area must maintain a

high standard of personal hygiene and appearance, be in good health, and adopt hygienic manufacturing principles. Personal hygiene refers to the cleanliness of a person's body. The health of workers also plays an important part in food sanitation. People are potential sources of microorganisms that cause illness in others through transmission of viruses or through food poisoning [29]. Personal hygiene means reasonably clean hands, forearms, neck, hair, and clothing that is liable to contact with food [26].

The education of food handlers is a crucial line of defense in the prevention of most types of foodborne illnesses [31]. Using questionnaires and microbial surveys, Aarnisalo et al. [14] investigated the hygienic working practices of maintenance personnel as well as the hygiene of the equipment in the Finnish food industry. The protective clothing, washing of hands and tools, and avoiding foreign bodies left on the production lines should be targeted when the hygienic working practices are developed for maintenance personnel. Based on the questionnaire to food processors, packaging machines, conveyors, dispensers, and slicing and cooling machines were considered the most problematic pieces of equipment hygienically mainly because of poor hygienic design. In order to improve food safety, both the training of maintenance personnel in food hygiene and equipment design should be more emphasized. Similarly, Nel et al. [32] interviewed workers from a deboning room of a high throughput abattoir by means of a structured questionnaire to ascertain the knowledge, attitude, beliefs, and practices regarding personal and general hygiene applied specifically in the deboning room. Basic hygiene practices were found to be in place and the workers adhered to the majority of these. The results highlighted a need for improved communication between management and workers as well as a need for more training in personal and general hygiene. Although basic personal and hygiene practices such as the wearing of overalls and gumboots, as well as the cleaning and disinfection of equipment are adhered to, they need to be optimized in order to be effective. The basic rules of personal hygiene are [1, 11, 27, 29]

i. Washing hands in hot water using plenty of soap and drying hands on a clean cloth or paper towel. Soap and water act as emulsifying agents to solubilize grease and oils on the hands. Increased friction through rubbing the hands together or using a scrub brush with the use of soap can reduce many transient and more resident bacteria than a quick handwash. Approximately 25% of food contamination is attributable to improper washing. Handwashing is conducted to break the transmission route of microorganisms from the hands to another source and reduce resident bacteria. Antimicrobial soaps are recommended for food preparation and handling areas. A fast-acting bactericidal skin cleanser kills most skin organisms on contact within 15 seconds. Proper washroom hygiene demands effective hand drying. A cloth towel system is hygienic, environmentally friendly, and cost effective. There is a lot more to skin cleaning than just soap and water. Inadequate or incorrect cleaning can lead to chronic skin disease. Skin cleaning must be done thoroughly, but also gently. The choice of cleaner depends largely on the type of contamination. The wrong choice could lead to skin irritation, dermatitis, and even more severe skin conditions.
ii. Washing hands after using the toilet, handling garbage or other soiled materials; handling uncooked muscle foods, egg products, or dairy products; handling money; smoking; or coughing and sneezing.
iii. Maintaining skin protection. Maintaining skin involves a three-point program: (i) correct skin protection for use before contact with various skin irritants and for under gloves; (ii) effective nonaggressive skin cleaning, which minimizes the loss of natural fats and oils from the skin; and (iii) after work, skin care to recondition the skin and support the healing of existing dermatitis or damage. A protective cream is also used in many cases. The types of materials handled dictate which type of protective cream to use. Protective creams do more than protect the skin. These functions include strengthening the outer layer of the skin, supporting the skin's ability to repair itself, stimulating skin growth, and make cleaning easier.
iv. Keeping fingernails short and clean, using a hairnet or cap, daily bathing, using deodorants, and washing hair at least twice a week.
v. Using protective clothing, footwear, and headgear, and these must be changed regularly. Beards must be kept short and trimmed, and a protective cover worn over them when considered appropriate by management.
vi. Avoiding use of nail varnish, false nails, and makeup; and avoiding wearing false eyelashes, wristwatches, and jewelry or ornaments. These items are places where more microorganisms can grow. These items are also difficult to clean.
vii. Avoid counting money during food handling.
viii. Avoiding consumption of food and drink in areas other than the tea bars and staff restaurant.
ix. Avoiding consumption of sweets and chewing gum in production areas.
x. Avoiding smoking or taking snuff in food production, warehouse, and distribution areas.
xi. Avoiding spitting in all areas on the site.
xii. Staying away from food when a person has an infected cut, boil, or other infection of the exposed skin; covering sneezes and coughs by means of a tissue or handkerchief.
xiii. Reporting all cases of diarrhea, fever, etc., and having periodic checkups.
xiv. Avoiding using bare hands to touch foodservice equipment and utensils. Disposable gloves should be used when contact is necessary.

All personnel should receive basic training in food handling and hygiene. This should include personal hygiene and an appreciation of the HACCP or GMP to implement HACCP. Management must select clean and healthy employees and ensure that they conduct hygienic practices. Sick workers should take sick leave and be properly treated before starting to work again.

Hand hygiene is the most important means of reducing the spread of infection. Peden and Vaughan [33] identified an improvement in the compliance rate of hand hygiene within a hospital when visual reminders, education, and positive reinforcement are used. Similarly, hand hygiene education and reminders to staff continue to maintain increased compliance and guarantee success [34]. Surveillance is a complex task that requires many elements to be addressed for data to be correctly reported. This includes the consistent application of data collection criteria by appropriately educated persons [35].

56.2.2.3 Cleaning of Factory Facility

Cleaning is an essential part of hygiene activities in a factory facility. Cleaning and disinfection prevent the contamination of food products from the factory facility and equipment surfaces. In the case of high-risk chilled foods, this is often a critical control point [30]. Recently, the usage of water became ever expansive and that economic reality must be acknowledged. Therefore, recycling water in food factories could be a solution to this problem. However, to apply this technique there must be a guideline to be followed. EHEDG and Codex Alimentarius have provided some hygiene legislation to this development, but it is not yet approved. Water recycling is still being evaluated in terms of consequences of the development, negative impacts that might be encountered, and emerging technical innovation that can be considered as a solution [36]. Napper [36] gave an example of removing organic material at the point where it comes into process water and before it gets contaminated with bacterial growth and chemicals. The observation was the level of chemical oxygen demand, which is the basis for cost of discarding of waste, which can be highly reduced. Moreover, this waste material can be also sold as a product. New technologies are proposed for disinfection, such as killing by adding metal ions to process water whether by chemicals or electrochemical process, and killing by creating chlorine and/or free radicals from tap water.

Cleaning and disinfection of surfaces in food factories is a big challenge. Titanium oxide added to the surface has a powerful oxidation ability that can be used to decompose materials, which can cause trouble even in small amounts. Titanium oxide is now widely used as a material for antibacterial tiles and in air-cleaning systems. When titanium oxide is coated on a glass, it shows it is superhydrophilic.

Effective cleaning is essential for the following purposes:

i. To remove materials on which foodborne pathogens might grow and spread easily through various carriers.
ii. To facilitate the effective disinfection process on the various compartments of a facility to reduce levels of foodborne pathogens.
iii. To prevent the risk of contamination/spreading of bad smell and odors, especially in the seafood and margarine industries.
iv. To promote a good image to visitors, including customers.
v. To offer a pleasant and hygienic working environment to industry workers.

56.2.2.4 Cleaning of Equipment

All equipment must be washed and rinsed. Frequent cleaning of plant equipment should be maintained at all times, and all parts should be continually washed by well-located continuous water sprays. Equipment should be cleaned as soon as possible after use, and disinfected or sterilized just before it is used again. If there is an appreciable time lag between uses, the equipment should be washed and disinfected or sterilized after use, and disinfected or sterilized again before use. The frequency will depend upon the hazard risk to the product being produced [5].

All equipment should be designed for easy cleaning. If the product is cleaned in place, the detergent used and the flow rates and angle or efficiency of the spray balls must be validated to ensure the soil is effectively removed from all areas with no blind spots. Hand cleaning depends upon operative training as well as the correct use of detergents [5]. All utensils used must be absolutely clean. Buckets, knives, and drain pans should be cleaned and rinsed whenever empty or not in use [1].

Evans [37] studied microbial contamination of food refrigeration equipment in chilled rooms in 15 plants processing a variety of foods (raw meat and salads, Chinese ready meals, dairy products, slicing and packaging of cooked meats, and catering establishments). An initial survey found microbes at a total 891 sites on evaporators, drip trays, and chilled room walls and was followed up with a more detailed examination of 336 sites with high counts, selecting for *Listeria* spp., coliforms, enterococci, *Staphylococcus aureus*, and *Bacillus cereus*. Although evaporator cleaning procedures were carried out in some factories as part of routine maintenance, these were not shown to be effective at maintaining low levels of bacteria on evaporators. To maintain evaporator hygiene, it was suggested that regular cleaning procedures, possibly by means of automated cleaning systems, should be considered [37].

Knowledge of adhering microflora on process equipment would also be valuable information in designing cleaning and disinfecting procedures, and is essential in the good hygienic practices program of food processing plants, as the development and design of improved cleaning and disinfecting procedures should target the microorganisms persisting and potentially contaminating the product. Many food pathogenic and spoilage bacteria are able to attach to food contact surfaces [38] and remain viable even after cleaning and disinfection [39]. Such adhered bacteria can detach during production and contaminated food as it passes the surfaces. Microorganisms attached to and growing on a surface are called microbial biofilms. The microbial fouling or biofouling causes problems in

the food industry. Hygienic problems in equipment are caused when microorganisms become attached to the surfaces and survive on them [40] and later become detached from them contaminating the product. Microflora adhering to the processing equipment during production and after cleaning and disinfecting procedures was identified in four different fish processing plants [41]. A total of 1009 microorganisms were isolated from various agar plates and identified. A stepwise procedure using simple phenotypic tests was used to identify the isolates and proved a fast way to group a large collection of microorganisms. *Pseudomonas*, Neisseriaceae, Enterobacteriaceae, coryneform, *Acinetobacter*, and lactic acid bacteria dominated the microflora of cold-smoked salmon plants, whereas the microflora in a plant processing semi-preserved herring consisted of *Pseudomonas*, *Alcaligenes*, and Enterobacteriaceae. *Psychrobacter*, *Staphylococcus*, and yeasts were found in a caviar processing plant. Overall, many microorganisms that are often isolated from fish were also isolated from the fish processing plants. However, some selection depending on processing parameters occurred, since halo- and osmo-tolerant organisms dominate in the caviar processing. After cleaning and disinfection, yeasts, *Pseudomonas,* Neisseriaceae, and *Alcaligenes* remained in smokehouses; yeasts and *Pseudomonas* in the herring plant; and *Pseudomonas*, *Staphylococcus*, and yeasts in the caviar plant. The dominant adhering organisms after cleaning and disinfection were pseudomonads and yeasts independently of the microflora during processing [41].

The ability of spores to adhere differed widely among strains [42]. All the members of the *B. cereus* group are surrounded by a loose balloon-like exosporium whose hydrophobic character could account for their strong adhesion to various materials such as stainless steel, polymers, and glass [43]. Transmission electron microscopy only revealed the presence of appendages [44, 45] on exosporium, but also that exosporium was made of a basal crystalline layer surrounded by a hairlike external layer whose filaments vary in length between species and strains [46]. Exosporium contains proteins, polysaccharides, lipids, and ash [47]. Some of the proteins constitutive of or associated with the exosporium were identified on *Bacillus anthracis* [48, 49] and *Bacillus cereus* spores [50]. A number of these proteins are not structural components but are closely associated with the exosporium, such as EA1 [48, 51], GroEL [48, 52], or InA [52]. Glycoproteins of high molecular weight (over 200 kDa) were identified in extracts from *B. anthracis* [49, 53], *B. thuringiensis* [54], and *B. cereus* [50, 52] spores. Only a few of the proteins and glycoproteins described in the literature have proven to be required for exosporium constitution or stability. Among the latter, BcIA is a structural component of the hairlike structure [53], while ExsFA and ExsFB are essential for the stability of the basal crystalline layer [55], and ExsA for the assembly of the coat and exosporium of *B. cereus* spores [56]. Some differences among spores from various *B. cereus* strains were previously evidenced. The most frequently reported variation is the surface energy, with *B. cereus* spores being moderately to highly hydrophobic [42, 57]. Additionally, the number and length of appendages could also differ among strains [44]. All these variations in spore surface properties could affect spore adhesion. Indeed, current theories on the mechanism by which bacteria adhere to inert surfaces suggest the importance of a cell surface's hydrophobic character [58, 59]. The presence of appendages, presumed to promote spore adhesion by overcoming the potential barrier and initiating contact with surfaces [60], was also believed to play a major role in attachment [2, 43, 61], but these results remained highly controversial [2, 45]. Last, the presence of some surface proteins and glycoproteins was known to play a major role in the adhesion of vegetative cells of various bacteria such as *Enterococcus faecalis* [62] and *Staphylococcus aureus* [63].

Tauveron et al. [64] examined seven strains of *Bacillus cereus* isolated from the environment and from patients with diarrheic symptoms. They were examined from two angles: (i) spore surface properties, and (ii) their ability to adhere to stainless steel and to resist a cleaning-in-place procedure. Their results revealed significant differences in their morphology (size of exosporium, length and number of appendages), hydrophobic character, and surface protein composition. Most of these proteins originated in the vegetative cell and were tightly bound to the external surface of the exosporium such as EAI or alanine racemase. Spore adhesion properties also varied from strain to strain. The ability to adhere was higher when spores were surrounded by long appendages, while the largest spores displayed the least resistance to cleaning. These observations suggested that food processing line contamination might be due to a given type of strain with specific surface properties (long appendages and small exosporium), which would represent an increased threat under the milder processing conditions required by consumers (minimally heat-treated foods for example) and by legal requirements (to limit effluents caused by hygiene procedures).

The dry-ice blasting technique has been widely employed in industry to clean equipment since the 1980s [65]. This process is a form of carbon dioxide cleaning at a temperature of –78.58°C in a pneumatic jet base and operates with dry-ice pellets as the single-way blast medium. The solid blast medium is environmentally responsible and no residue is left after the cleaning process since the dry ice directly sublimates when subjected to the surface of the equipment [66]. The production of the blast medium from other industrial processes is a by-product from industries such as ammonia production, oil and gas refineries, and ethanol production, therefore, no additional greenhouse gases to the atmosphere. This type of cleaning media is approved by the U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration (FDA), and U.S. Department of Agriculture (USDA). This technique is also used as a preservative since it leaves behind no toxic residues in food. Moreover, carbon dioxide can act as a refrigerant in modified atmosphere packaging during transport and storage. Further, it inhibits bacterial growth as the dry ice sublimates by displacing the oxygen required by aerobic organisms and lowering the pH of food to bacteriostatic levels by forming carbonic acid [67]. Witte et al. [68] investigated the disinfection potential of dry-ice blasting and parameters

influencing cleaning efficacy. This method proved to remove bacterial cells to a similar extent from several surfaces and components of dairy production equipment, occasionally with a slight abrasive effect. The bacterial removal rate was less than 5 $\log_{10}$ units, therefore, they did not recommend dry-ice blasting as a disinfection method, but it can be used as an efficient cleaning comparable to other conventional methods. In general, dry-ice blasting is regarded as being environmentally friendly and requires less energy and less water [69].

56.2.2.5 Hygiene Monitoring

The evaluation of the effectiveness of a sanitation program may be carried out in several ways: visual inspection, swabbing, and microbiological analysis, contact plates, or ATP (adenosine triphosphate) bioluminescence [30]. Traditional hygiene monitoring using culturing techniques creates a number of difficulties, including it is laborious and time-consuming. Estimation of the total colony count may take 24–48 h. If it is necessary to detect or estimate the presence of specific microorganisms, 4–7 days may elapse before a result is obtained. Results need to be generated sufficiently quickly to take remedial action or to prevent contaminated surfaces from being reused [30]. The technique of ATP bioluminescent involves taking a sample by swabbing a surface and then processing the swab. More details are given by Hawronskyi and Holah [30] and Griffith et al. [70]. These methods can be divided into three different types: cuvette-based, pen-based, and swab-based. The speed of this method enables remedial action to be taken in the case of poorly cleaned equipment. The disadvantages are that (i) it cannot currently give any indication of the presence or absence of pathogens, only a total level of ATP contamination on a surface; (ii) the presence of detergents or other chemicals may interfere with bioluminescent reaction; and (iii) it is expensive compared to the low-cost conventional culture techniques [30].

56.3 CONCLUSION

Hygienic and sanitary design of a food facility is essential during construction and renovation to ensure effective hygienic operations. A functioning sanitary design begins with the selection of a best-suited site that is free of environmental hazards such as polluted air, pests, and foodborne pathogens. An appropriate site planning with effective sanitary principles, such as having raw material and final product storage, and a proper drainage system and waste disposal, can reduce contamination from the environment. All other aspects of sanitary design and operations such as equipment design, good manufacturing practice (GMP), HACCP, personal hygiene and food handling, cleaning of the factory facility, cleaning of equipment, and hygienic monitoring must be followed thoroughly to offer a hygienic environment and ensure food safety. However, it is essential to install a hygienic flow diagram through the production and processing lines that facilitates the safe production of a final product by preventing contact with raw materials and unprocessed items.

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57 Hazard Analysis and Critical Control Point (HACCP)

Titus De Silva

CONTENTS

57.1 BACKGROUND

57.1.1 Need for an Effective Food Safety Assurance System

Food safety has been of concern since the Middle Ages, and regulatory measures have been enforced to prevent the sale of adulterated or contaminated food. Many rules and recommendations advocated in religions or historical texts are evidence of a concern to protect people against foodborne diseases and food adulteration. Motarjemi et al. [1] summarized the present concern and emphasized the need for an effective food safety program [1].

Technological progress in food processing has brought about greater awareness of problems associated with food preservation. With the expansion of global food trade, food safety has become a worldwide issue. According to the World Health Organization (WHO), "thousands of millions" of cases of foodborne illnesses occur every year and two million children die each year from diarrhea. These cases relate to the microbiological contaminants only, and when chemical and physical contaminants are taken into consideration, the number of cases is much higher [2].

International food trade contributes to the spread of foodborne illnesses in five ways [2]: (i) new threats arise as a result of the introduction of pathogens and contaminants from one country to another, (ii) pathogens that have been controlled in one country can be reintroduced in another country where they have not been controlled, (iii) transportation over long distances and long periods of time provides ample opportunities for contamination and growth of microorganisms, (iv) contaminant food can reach more people resulting in major outbreaks of disease, and (v) the history of the food product may be unknown by the importing country when trans-shipping of food through one or more countries takes place. International and national organizations have enforced laws and regulations to achieve quality and safety in food preparation and preservation in order to safeguard the consumer from foodborne infections and intoxication.

57.1.2 The Development of HACCP

The principles of the Hazard Analysis and Critical Control Point (HACCP) technique were applied to the chemical

processing industry, particularly in Great Britain, more than 40 years ago [3]. The Pillsbury Company, together with NASA and the US Army's Research, Development and Engineering Center at Natick, first developed this system to ensure the safety of astronauts' food [1, 4, 5]. Since then the HACCP system has grown to become a universally recognized and accepted method for food safety assurance. The World Health Organization (WHO) has recognized the importance of the HACCP system for prevention of foodborne diseases for over 20 years and has played an important role in its development and promotion. One of the highlights in the history of the HACCP system was when in 1993 the Codex Guidelines for the Application of the HACCP system were adopted by the FAO/WHO Codex Alimentarius Commission, requiring them for international trade [6]. In the United States, large chemical industries have also adopted hazard control programs. The U.S. Occupational Safety and Health Administration (OSHA) introduced the HACCP technique to reduce accidents.

57.1.3 Benefits of HACCP

Lack of food safety systems costs the food industry millions of dollars annually through waste, reprocessing, recalls, and resulting losses of sales. Foodborne diseases are no longer limited to developing countries. In the United States, the Centers for Disease Control and Prevention (CDC) report that foodborne diseases account for 76 million illnesses, 325,000 hospitalizations, and 5000 deaths each year. According to the World Health Organization, the cost of just five foodborne diseases in England and Wales was estimated at £300–700 million in 1996 [2].

It is now recognized internationally that the most cost-effective approach to food safety is through the application of the HACCP technique. By adopting an effective food safety system based on HACCP, the industry can minimize the potential for things to go wrong and ensure the safety of food products. HACCP is entirely complementary and adds essential safety elements to existing processing systems such as Good Manufacturing Practices and ISO 9000 standards. Food producers as well as retailers and consumers will benefit as a result of an effective HACCP program. The benefits of the HACCP system are summarized in Table 57.1 [1].

57.2 TERMINOLOGY

The terms, such as hazard, severity, risk, and hazard analysis, that are important to the HACCP concept are defined in Table 57.2. It is important to understand the terminology first before applying HACCP in food systems.

57.3 MANAGEMENT COMMITMENT

The design and implementation of a HACCP system is the responsibility of the HACCP team. But the senior management must be aware of the fundamental requirements of the food safety program so that they can commit the necessary resources and have a positive approach to the project. Its role then is to influence the project's success by creating a "buy-in" from every member of the organization. In the absence of total commitment from the senior managers, the organization runs the risk of failure at a critical point [7]. The senior management must acknowledge that a HACCP program is a comprehensive tool that helps everyone in the organization to maintain a safe food production and serving environment. As with a QMS, the food safety program involves suppliers, staff, and customers. In addition, the organization has to comply with regulatory requirements. Therefore, support from the senior management team, regulatory bodies, training institutions, and the industry are all essential in designing an effective food safety program. The suppliers can support the organization's commitment by recommending equipment and procedures that will assist in realizing the long-term food safety goals [8].

57.4 PREREQUISITE PROGRAMS

Prerequisite programs are an essential part of the HACCP program. It is not possible to implement a HACCP program without the prerequisite programs in place. These provide the basic environment and operating conditions that ensure the production and delivery of safe food. The National Advisory Committee for the Microbiological Criteria for Foods [9] has recommended the prerequisite programs shown in Table 57.3 as a foundation for an effective HACCP system in the organization.

Other prerequisite programs might include (i) quality assurance procedures, (ii) product formulations, (iii) glass control,

TABLE 57.1
Benefits of the HACCP System

Ensures safety of food products through preventive measures rather than through final inspection and testing
Capable of identifying all potential hazards
Easy to introduce technological advances in equipment design and processing procedures related to food products
Directs resources to the most critical part of the food-processing system
Encourages confidence in food products by improving the relationships among regulatory bodies, food processors, and the consumer
Promotes continuous improvement of the system through regular audits
Focus on safety issues in the whole chain from "farm to fork"
Complements the quality management system (e.g., ISO 9000)

TABLE 57.2
Terminologies Related to HACCP

Term	Definition
Hazard	A biological, chemical, or physical or other property in a food product that has the potential to harm the consumer or cause illness. It can occur in the ingredients or at any stage in the life of a product. The term can thus be applied to foreign material, chemical residues, and/or microbiological contamination.
Severity	The seriousness or consequence of exposure to the hazard.
Risk	The estimate of the probability of a hazard occurring.
Hazard Analysis	The identification of biological, chemical, or physical and/or other hazards associated with ingredients, production practices, processing, storage, distribution, retailing, and use.
Critical control point (CCP)	An operational step in a manufacturing process that results in injury or risk to consumer if not controlled. At critical control points, controlling measures can be exercised to eliminate or minimize any form of hazard.
Control point	An operational step in a manufacturing and distribution process that may be controlled in order to maintain quality and meet regulatory requirements.
HACCP plan	A document that sets out the procedures based on the principles of HACCP to be followed to assure food safety.
Hazard Analysis and Critical Control Point (HACCP)	A scientific, rational, and systemic approach to identification, assessment, and control of hazards during production, processing, manufacturing, preparation, and use of food to ensure that it is safe when consumed. The HACCP system provides a preventive and thus a cost-effective approach to food safety.
HACCP system	The organizational structure, procedures, processes, and resources needed to implement the HACCP plan.
Critical limit	One or more prescribed tolerances that must be met to ensure that a CCP effectively controls a health hazard.
Sensitive ingredient	An ingredient known to have been associated with a hazard and about which there is a concern.
Validation	A review of the HACCP plan to ensure that all elements of the plan are accurate and correct.
Verification	The use of methods, procedures, and/or tests to ensure that the requirements of the HACCP system have been fulfilled.

TABLE 57.3
Prerequisite Programs

	Prerequisite Program	Scope
1	Facilities and premises	Suitability of production environment including buildings, pathways, drainage, waste management, etc. Provision of adequate space for manufacture, storage, cooling, and refrigeration Provision of ventilation, water supply, and lighting facilities for personnel
2	Supplier control	Program for the approval of suppliers Effective GMP and food safety programs
3	Specifications	Written specifications for all ingredients, packaging, and processes
4	Equipment	Calibration of equipment Preventive maintenance according to an established schedule
5	Cleaning and sanitation	Validation of sanitation methods Availability of documented procedures Regular cleaning and sanitation of equipment
6	Personnel hygiene	Establish a personnel hygiene policy Ensure that all employees follow the required personnel hygiene policy
7	Training	Management of training records in personnel hygiene, GMP, cleaning, sanitation procedures, personnel safety, and their role in the HACCP program
8	Management of chemicals	Proper and safe storage and segregation of food and non-food chemicals
9	Receiving, storage, and transport	Storage of all raw materials and products under sanitary conditions Maintaining appropriate environmental conditions for storage
10	Traceability and recall	Batch coding of all raw materials and products Establish an effective recall procedure
11	Pest control	An effective pest control program

(iv) labeling, and (v) employee food and ingredient handling practices. An HACCP program ensures that the food is safe to consume, and the integration of the prerequisite programs provides assurance that food is safe as well as wholesome. The prerequisite programs have to be regularly audited and reviewed just like any other management program. While these programs can be managed separately from the HACCP program, some aspects such as recall procedure, preventive

maintenance, sanitation, and hygiene may be incorporated into the HACCP plan.

57.5 THE SCOPE OF THE HACCP PROGRAM

In the early years following the introduction of HACCP, concern was focused on microbiological integrity. The basic philosophy was to examine the food ingredients as they are processed until consumption of the finished product. Aggressive competition required organizations to reduce costs while maintaining quality. Increasing consumer awareness and legal liability to produce safe food forced organizations to adopt a broad view of the food safety program. There were other significant changes. Changes in process technology, increased automation, complex packaging solutions, new ingredients and improved formulations, greater emphasis on sensory evaluations, and complex distribution networks leading to reduced delivery times had a major impact on food safety requirements.

The generic requirements of food safety were stipulated in the Codex Alimentarius which is a collection of food standards adopted by the Codex Alimentarius Commission [10]. The HACCP program incorporates the general principles of food hygiene as well as appropriate commodity standards. In 1998, the British Retail Consortium (BRC) developed and introduced the BRC Technical Standards and Protocols for supplying retailer branded food products. Although it was originally developed primarily for the supply of retailer branded products, in recent years it has been used as a basis for developing HACCP programs. It incorporates the management system of ISO 9000, principles of Codex Alimentarius, and Good Manufacturing Practices (GMP) [11]. Thus, a complete HACCP program includes (i) HACCP principles, (ii) management principles, (iii) plant environment standards, (iv) process and product control, and (v) personnel hygiene.

57.6 THE SEVEN PRINCIPLES

The seven principles of HACCP were formulated by the National Advisory Committee on Microbiological Criteria for foods (NACMF) in 1987 [12]. They are now widely accepted as the standard for developing a food safety program.

1. Analyze the hazards and assess the risks.
1. Identify the CCPs.
3. Define critical limits for each CCP.
4. Establish controls to monitor the CCPs.
5. Establish corrective actions.
6. Define record keeping and documentation requirements.
7. Establish verification procedures.

These seven principles form the framework of the HACCP program, and these will be described in the sections that follow. In the future, it is possible that three more principles will be added [13] to the seven already in place: (i) summary and interpretation of each HACCP system (this review briefly summarizes the system, indicates any remaining hazards, and assesses the risks of occurrence), (ii) education and training, and (iii) validation.

57.7 DEVELOPMENT OF THE HACCP PLAN

The development of the HACCP plan goes through four distinct stages. Table 57.4 shows the key tasks associated with each stage. These tasks are applicable to any food processing operation regardless of the size. The program evolves as the HACCP team works through these stages [14]. It is a good idea to carry out a HACCP study for each new product in each plant. Hazard analysis critically examines the quality of all ingredients, processing steps, and the product itself. The CCPs can be identified by analyzing the hazards in each processing step. The HACCP plan is managed by regular monitoring and reviewing of the system through the implementation of corrective action when necessary.

57.7.1 The Team

The HACCP study begins with the selection of a team consisting of members drawn from various disciplines in the food-processing operation. The success of the HACCP program depends on the constitution of the HACCP team [15]. The selection of the team is an important step in the development of the HACCP program. It is not a management team, but a team drawn from all levels depending upon the expertise and experience required. All of the key functions of the organization should be represented in the team, and it may include a project leader, a production manager, a technical expert, an engineer, a secretary, and others as required. Once the team is formed the members will require further training on HACCP principles, and the members should be provided with the necessary tools to perform the tasks. Its objectives [16] will be to (i) define the type of food produced by the organization, (ii) identify how food product ingredients are received, (iii)

TABLE 57.4
Development of the HACCP Plan

Stage	Key Tasks
1	1 Assemble the team
	2 Train the staff
	3 Describe the product and intended use
2	4 Draw the flow diagram
	5 Analyze the hazards (Principle 1)
	6 Establish the CCPs (Principle 2)
	7 Define critical limits (Principle 3)
	8 Establish monitoring methods (Principle 4)
	9 Establish corrective actions (Principle 5)
3	10 Define verification methods (Principle 6)
	11 Establish documentation and record keeping requirements (Principle 7)
	12 Validate the HACCP plan
4	13 Review and improve the program

TABLE 57.5
Responsibilities of HACCP Team Members

Team Member	Responsibility
Project leader	Convenes and chairs meetings
Production manager	Constructs flow charts
	Advises on production issues and process capability
Technical expert	Advises on technical issues
	Identifies hazards and recommends solutions
Engineer	Supplies information on performance of equipment and machinery
	Makes recommendations on new machinery, equipment, or processes that may be required
Secretary	Records proceedings of meetings
Others (as required)	Provide information on specialist areas

define the processes and their controls, (iv) identify how the food is stored and distributed, and (v) identify the hazards and the CCPs. In the absence of in-house expertise, it may be necessary to seek the advice of external consultants. The team leader or coordinator has a significant impact on the outcome of the program, and he/she is responsible for assigning various tasks to the members of the team. The responsibilities of the team are summarized in Table 57.5.

57.7.2 Training

HACCP training has now been accepted as the most cost-effective means of controlling hazards related to microbiological, physical, and chemical contamination of foods. The implementation of the HACCP plan is a team exercise. Training and education are essential if the full benefits are to be achieved [17]. Food producers have the responsibility to produce safe food, and the regulatory bodies need to be competent in monitoring the HACCP programs.

HACCP training should provide (i) knowledge of concepts, principles, and benefits of the HACCP program, (ii) practical skills and knowledge necessary for the implementation of the HACCP program, and (iii) the skills needed for further development of the HACCP program. Regulatory authorities, senior managers, shop floor personnel, and technical managers should be involved in the HACCP training program. The training program itself should target the needs of each of the above groups. For example, regulatory authorities need to have knowledge of the concepts, principles, and benefits of the HACCP programs, and shop floor personnel need to have practical skills. Scientific data should be used to support the training progress. Software on HACCP programs is now available, and the material presented in such programs can be an essential tool for training.

57.7.3 Product Description and Intended Use

The HACCP team needs to have a complete understanding of the product, its intended use, the ingredients used, the composition of the product, and the processing steps. It is necessary to have this information before the analysis of hazards because the food products have to be assessed in relation to the ability of different pathogens to grow. The product description [18] should include (i) name of the product, (ii) end-product features (e.g. pH, preservatives, etc.), (iii) how the product is to be used (i.e. ready-to-eat, further processing, heated prior to consumption), (iv) packaging details (e.g. packaging materials and packaging conditions), (v) shelf-life, (vi) distribution outlets, (vii) labeling instructions, (viii) shipping conditions, and (ix) target consumer group. An example of a product description is shown in Table 57.6.

TABLE 57.6
Product Description of Country Medium Wine

Product Feature	Description
1. Product name	Country Medium Wine
2. Product features	Alcohol 12%v/v, pH, preservatives, SO_2
3. How the product is to be used	Cool before serving
4. Packaging	3-liter plastic bags of special composition which prevent the entry of O_2
5. Shelf life	9 months from the date of packing
6. Distribution outlets	Retail shops
7. Labeling	Alcohol content, preservatives, storage conditions, and the number of standard drinks
8. Shipping conditions	No physical damage or extremes of temperature
9. Target consumer group	None

TABLE 57.7
List of Raw Materials Used in the Production of Wine

Primary Ingredients	Chemicals	Packaging Materials	Non-Contact Packaging
Grapes	See attached list (attach list of chemicals used)	Bottles, glass	Cases, corrugated board
		Bottles, plastic	Labels
		3-liter bags	Muselets
		Metal caps	Shrouds
		Plastic caps	Capsules, PVC
		Corks	Sections, corrugated board

The product ingredients include raw materials, processing aids, and packaging material. Regulatory requirements have to be checked to ensure that the additives and preservatives are permissible and are within specified limits. An example of a list of ingredients and packaging materials is shown in Table 57.7.

The team has to gather further information on processing steps and the formulations used in the production of food. A checklist may include but is not limited to the following: (i) possibility of contamination during preparation and storage, (ii) inactivation of microorganisms and toxins and the possibility of microbiological, chemical, and physical contamination during processing, (iii) pH, water activity (a_w), and/or reduction/oxidation potential, (iv) possibility of the presence of microorganisms in raw materials and packaging, (v) basis of process technology, and (vi) effect of packaging on the survival and/or the growth of microorganisms.

57.7.4 Flow Diagram

A food-processing operation is a continuous process of flow of food ingredients from "farm to fork" (growing, harvesting, receiving, storage, processing, packaging, transportation, and service). A flow diagram breaks up the process into a series of sequential steps that assist further evaluation. It typically commences at the point where the food is received at the operation. Thus, the steps in the flow diagram typically include the entry of ingredients, all processing steps, packaging, storage, distribution, and handling by the consumer. In a simple flow diagram, the process steps are shown inside boxes with arrows indicating the entry and exit points.

In a large food production operation, it is not possible to draw flow diagrams for each of the recipes. However they could be grouped according to the method of processing, for example, baking, cooking, chilling, steaming, cold preparations, etc. Yet another approach is to group recipes according to the category of food—thin cooking, thick cooking, sauces and brews, fruits and vegetables, starches, breads and batter, cold combinations, and hot combinations. In such instances, a single flow diagram would be adequate for each food category [19]. Figures 57.1 and 57.2 show the flow diagrams for the production of chicken and vegetable salad and hot smoked salmon, respectively.

57.7.5 Types of Hazards

Hazards in food processing can be classified into three types: Biological, chemical, and physical.

57.7.5.1 Biological Hazards

Biological hazards are associated with microbiological organisms such as bacteria, viruses, fungi, and parasites. Most foodborne illnesses are caused by pathogenic bacteria, and a certain level of microorganisms occurs in some raw foods. Viruses can be foodborne/waterborne or transmitted by human, animal, or other contact. They survive on living cells and therefore cannot replicate in food but can be carried by it. Parasites are host-specific but can include humans in their life cycle. They occur in undercooked meat products or contaminated ready-to-eat food. Fungi include molds and yeasts, and some produce mycotoxins which are toxic for humans and animals. The survival and the growth of microorganisms depend upon several factors: (i) inadequate time–temperature combination during heat treatment, (ii) slow acid production during fermentation, (iii) holding foods between 7 and 65°C because of processing delays, (iv) storage of foods for long periods at refrigerated temperatures, (v) high water activity (a_w), and (vi) low levels of chemical preservatives

57.7.5.2 Chemical Hazards

Chemicals are used in food products, such as pesticides during the growing stage or as food additives during formulation and processing stages. Types and concentration levels of chemicals are important for safety aspects. Some examples of hazardous chemicals are heavy metals such as lead, tin, cadmium, copper, and mercury; food additives such as certain preservatives, colorings, and conditioners; and others such as solvents, cleaning agents, paints, and additives.

57.7.5.3 Physical Hazards

Physical hazards are caused by foreign matter that can enter into a food product at any stage from the processing of raw materials to the consumption of the finished product. Foreign matter may be visible to the naked eye or may be dissolved or dispersed in the food product. The physical form of the foreign matter can vary from powder to particulate matter, depending upon its type and origin. The detection of foreign matter in a food product is not easy because of the variability and

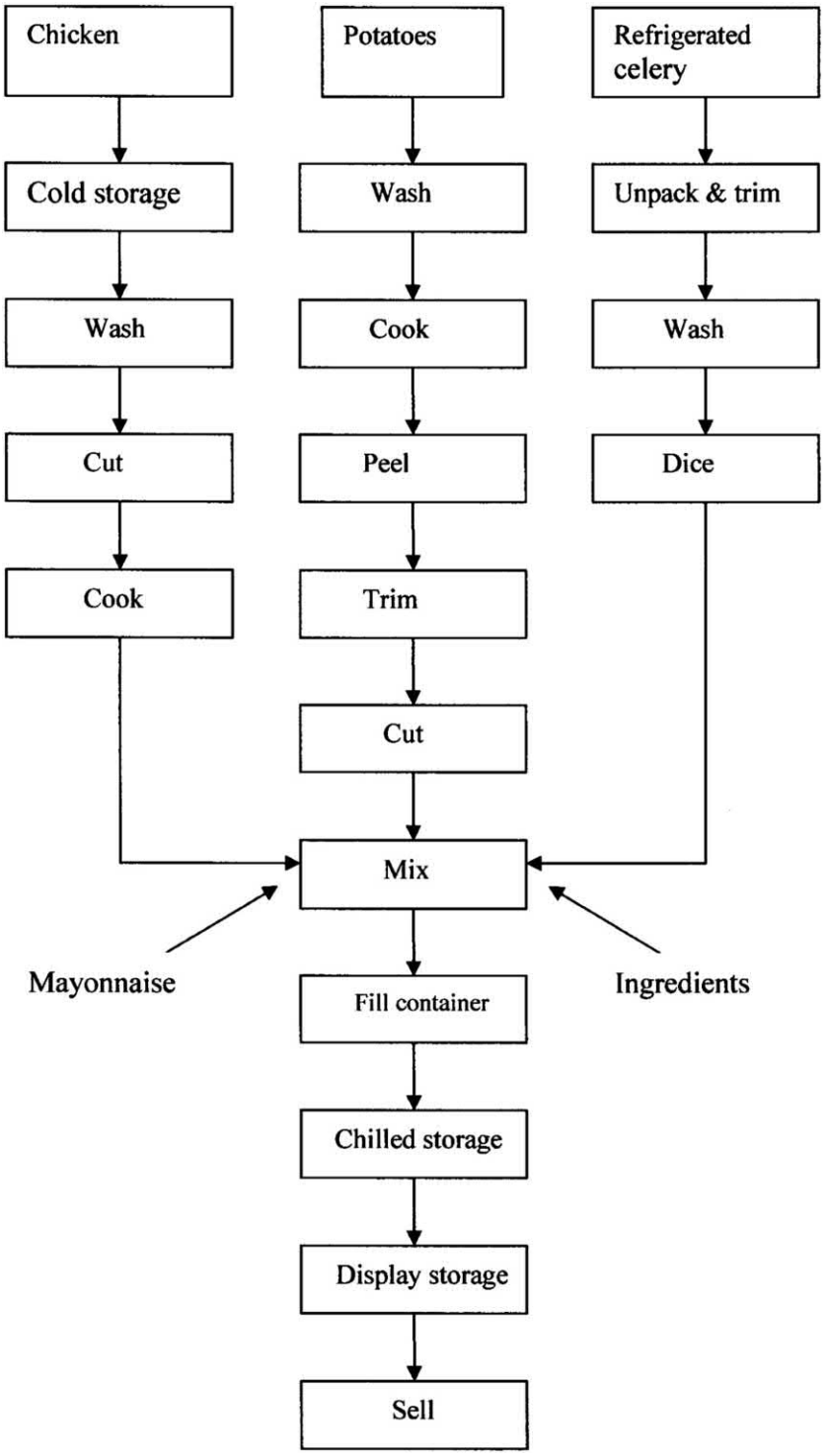

FIGURE 57.1 Flow diagram for the production of chicken and vegetable salad.

infrequent occurrence. Some of the common types of foreign matter associated with physical hazards in food are insects, spiders, worms, etc. (although these organisms are nonhazardous in themselves, they may carry pathogenic microorganisms), parts of animals, birds, metals, machine parts, glass pieces, plastic objects, sand, stones, dirt, cigarette butts, and plastic dressing strips.

57.7.6 Sources of Hazards

57.7.6.1 Raw Materials

Raw materials are the primary source of contamination. Failure to follow basic quality assurance procedures on raw materials may lead to food products that are unsafe for consumption. The common quality assurance procedures carried

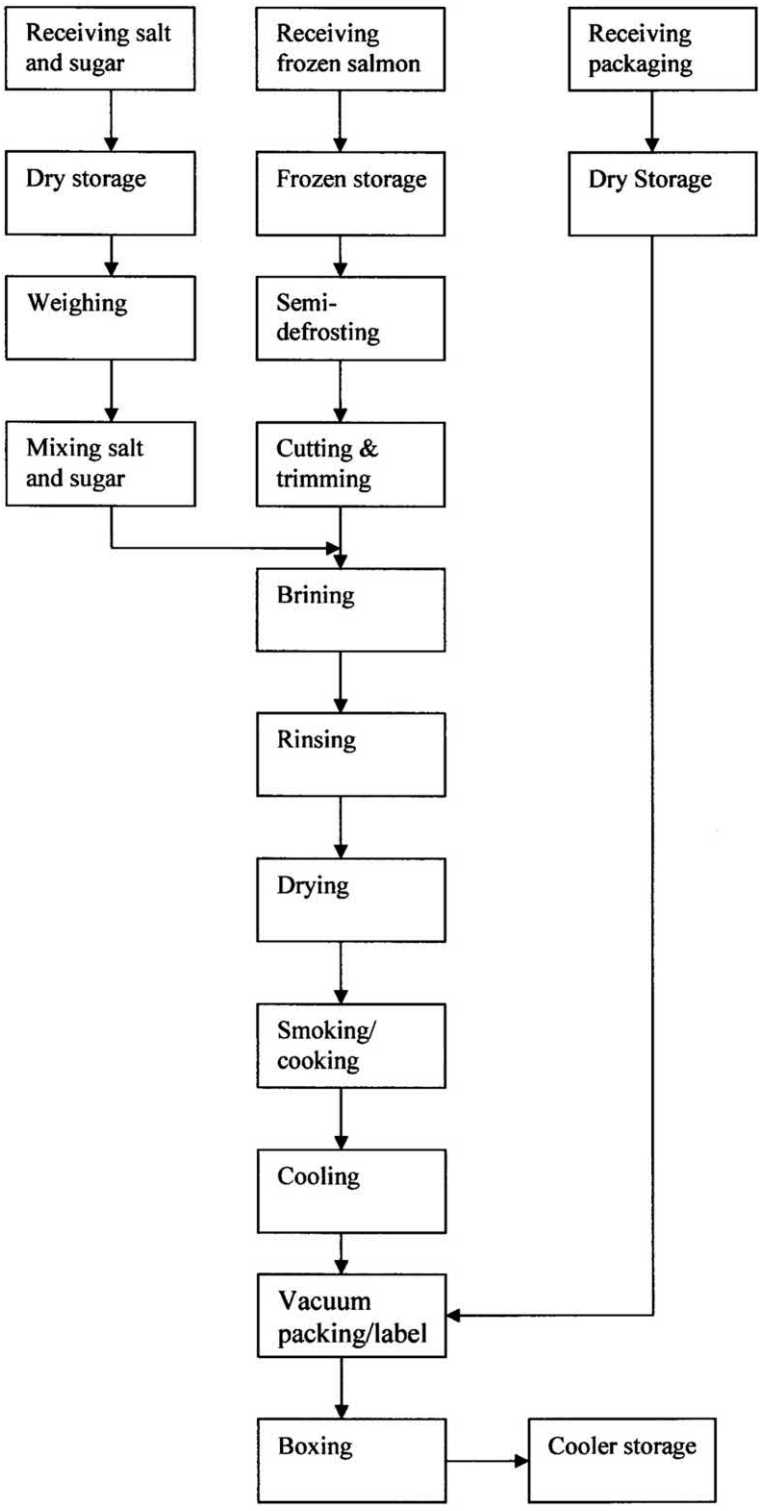

FIGURE 57.2 Flow diagram for the production of vacuum-packed hot smoked salmon.

out on raw materials are related to (i) identification and labeling, (ii) storage conditions, (iii) handling requirements, (iv) preparation and processing, and (v) isolation of unsuitable raw materials. The raw materials that are most likely to cause microbial hazards are meat, poultry, fish, and dairy products. The level of microbial contamination depends upon (i) the source, (ii) the refining and handling process, (iii) the packaging material, and (iv) the storage conditions [20].

The HACCP technique has been closely associated with the protection of the consumer from microbiological hazards. Such a narrow focus has been criticized on the grounds that microbiological hazards account for only one type of hazard

[21]. Raw materials may carry traces of chemicals and foreign matter. Heavy metals such as lead, arsenic, mercury, tin, and cadmium are thought to be of greatest health concern. They occur in vegetables grown in contaminated soils. Packaging materials such as lead capsules in wine bottles and solder from the side seams of cans are potential sources of contamination. Adhesives, coatings, and resins used in packages may cause health hazards unless they comply with the health and safety requirements [22]. Seam defects in cans and seal defects in other packaging designs can lead to microbial contamination.

Cleaning chemicals, solvents, lubricants, and dirty or incompletely washed bottles may contaminate food products. Excessive amounts of sulfur dioxide used in the sterilization process prior to filling bottles result in high levels of sulfur dioxide in the food product (e.g., high sulfur dioxide levels in wine). Preservatives, colorings, flavors, and conditioners are common additives used in food processing, and excessive amounts of these can be harmful. The permissible levels of food additives are governed by the food regulations in each country. Fruits and vegetables sprayed with pesticides can retain high levels of pesticide residues unless the applications are carefully monitored. Foreign objects such as stones and insect parts may also be found in fruits and vegetables. An unhygienic working environment can promote contamination with rodent bait, insecticides, insects, etc. Other sources of chemical and foreign body contamination include glass fragments from broken light fittings and thermometers, stones and particles of wood in sun-dried fruits, herbs, and spices, and accidental contamination with cleaning chemicals or other toxic non-food substances [23].

57.7.6.2 Processing Steps

Uncontrolled processing operations can lead to hazardous situations. Failure to maintain processing conditions such as temperature/time, delay in processing, using incorrect formulations and procedures, and following unauthorized processing techniques may all result in contamination and/or microbial growth. Mercury thermometers in the processing area can be a potential hazard, and most industries prohibit the use of such thermometers in their factories. Poor cleaning practices may leave excess cleaning chemical residues on plant and equipment.

57.7.6.3 Machinery

Unclean and unhygienic equipment can easily promote the growth of microorganisms or other hazards. Failure to maintain the sterility of equipment when it is required results in microbial contamination. Proper setting up of equipment should also be followed. Glass bottles can get chipped at the filler or capping machine if the machines are not properly set up. Imperfectly made containers can also contaminate the food with the material of the container. Metal pieces from meshes or metal parts and nuts and bolts can easily get mixed with the food product if the machines are not regularly maintained. Badly designed or modified plant and equipment can promote the growth of microorganisms in dead-ends of pipework or dirt-traps.

Preventive maintenance of machinery is an important aspect of a safety-management program. If safety requirements are ignored, the layout of machinery and equipment can be a potential hazard. The machinery should be examined at intervals to ensure safe operation. Any change in engineering should be such that it is not hazardous.

57.7.6.4 Handling of Food

With the introduction of highly automated, high-speed machinery, vast volumes of food products are processed, stored, and transported to distribution centers and retail chains. Therefore, food safety depends upon processing characteristics as well as handling during transport, storage, and customer use. Hazards may develop due to inadequate temperature control during storage, transportation, retail handling, and home storage. Products such as chilled/frozen entrees and meal components are preserved by refrigeration. Hazards could be developed if these products are stored at higher temperatures or used beyond their recommended shelf life [24].

Failure to rotate stocks of dated products can result in outdated products reaching the consumer. Abuse by the customer is possible in the absence of clear storage or preparation instructions. Lack of knowledge about handling, cooking, and storage of foods increases the risk of hazard occurrence. Personal hygiene is extremely important in any food-serving establishment. If adequate precautions are not taken, food handlers can transmit pathogenic bacteria. Personal articles such as jewelry can get mixed with foods during preparation.

57.7.6.5 Environmental Conditions

Hazards due to environmental conditions may affect raw materials, processing, and machinery. Pollution of water and soil can have alarming results through the food chain. Through regulatory requirements, most countries monitor and control the disposal of domestic and industrial wastes to prevent the entry of hazardous materials and rodents into the food production environment. Environmental contamination may also be due to foreign matter, chemicals such as sprays, and contaminants in water.

57.7.7 Some Measures for Controlling Hazards

57.7.7.1 Measures at the Processing and Packaging Stages

57.7.7.1.1 Raw Materials

In a food-processing environment, raw materials constitute one of the most important areas that must be carefully controlled. The food producer has no direct control over the quality of incoming raw materials. Until adequate control can be established over the entire range of raw materials to prevent the entry of or eliminate harmful or potentially harmful organisms and residues, constant vigilance must be maintained. This is particularly true of "sensitive ingredients"—those ingredients that have been historically associated with a known hazard, such as eggs, fish, milk, cheese,

TABLE 57.8
Processing Methods to Control Microorganisms in Raw Materials and Packaging

Method	Control Parameter
Heat treatment	Time, temperature, humidity
Filtration	Pore size, filter integrity
Irradiation	Dosage and density of load
Chemical	Concentration, pH, temperature

shellfish, etc. The processes used to remove or destroy microorganisms in raw materials and packaging are shown in Table 57.8 [20]. Some of the controls that can be established in order to ensure that incoming raw materials do not cause a health hazard are summarized in Table 57.9.

Packaging materials can also be a source of health hazards since most users are unaware of the materials used. Controls can be established by specifying the recommended types of packaging materials. Bulk containers that are used to transport food products should have a cleaning program in place, which should be audited. Only permissible products should be transported in bulk containers that are used to carry food products. Other controls include tamper-proof seals, inspection of samples on delivery, and maintaining appropriate storage conditions.

57.7.7.1.2 Processing Steps

A wide variety of methods can be employed to control the hazards that may be encountered during the processing operations. The type of control mechanism depends upon the processing method or methods employed in the plant. Temperature and pressure recorders are common in most food-processing systems. With advances in electronic technology, thermometers are not often used, and if they are employed, mercury thermometers should be avoided. Control charts, log sheets, and other records can be employed to monitor the temperature and pressure in a processing environment. Batch records should clearly state the type and quantities of ingredients used in production. Products that require a use-by date should be controlled at the source. All products including the ingredients used should have batch number or a lot number to enable traceability. Finished products should be maintained at the specified temperature, and products held under quarantine should be clearly labeled to prevent them from being dispatched. Operating storage tanks at positive pressure can create problems of cross-contamination between liquid and gas lines. Such cross-contamination can be avoided by using nonreturn valves at appropriate locations. Changes made in a process should be controlled through a change control procedure, which should include a reassessment of the hazards and CCPs.

57.7.7.1.3 Plant and Machinery

Hazards due to the plant and machinery can be controlled by the development and maintenance of physical equipment and accessories used to manufacture a food product. It is necessary to thoroughly clean and sterilize all equipment and utensils before and after processing. It is also important to recognize the significance of HACCP principles in planning and the layout of engineering equipment. A hazard control program requires that every production line in the plant be correctly laid out showing the operation and interrelation of all machinery and equipment. A preventive maintenance program should be in place that indicates the frequency at which equipment should be checked. When a change in machinery or machine settings occurs, the hazards must be reassessed. Critical measuring equipment such as thermometers, weighing scales, etc. should be calibrated by organizations authorized by the national bodies to do so, so that the measurements can be traced to a national standard. Line lubricants, grease, and chemicals used for cleaning of equipment should be recognized as safe and should be purchased from an approved supplier. The operators are the closest to the machinery, and they should be adequately trained to identify potential hazards. Unusual observations should be immediately investigated.

57.7.7.1.4 Storage and Distribution

Hazards due to storage, dispatch, and distribution are associated with storage conditions, stock rotation, and physical

TABLE 57.9
Control of Incoming Raw Materials to Ensure Safety

1. Be highly selective of sources and suppliers of materials and their ability to produce and deliver a safe product consistently by implementing an approved supplier policy.
2. Establish specifications for raw materials taking into consideration those characteristics that are critical to quality and safety.
3. Avoid using the cheapest price as the sole criterion for purchase. Relate the price to risk assessment.
4. Review any new ingredients introduced into the system. Instruct the supplier to inform you of any changed characteristics of the raw material, since even minor changes may affect the final quality.
5. Carry out periodic audits at the supplier's premises.
6. Instruct the supplier to have a HACCP and a QA program in place. Provide encouragement and support if necessary. Developing a partnership can be mutually beneficial.
7. Inform the supplier to label the raw materials accurately and provide assurance in the form of compliance certificate when they are delivered.
8. Carry out periodic tests on raw materials on a random basis on delivery.
9. Monitor storage conditions of raw materials both at the supplier and the producer.
10. Encourage the raw material suppliers to develop safe packaging of ingredients.

locations. All purchased products should be handled and stored in a manner to ensure protection, preservation, and freedom from contamination, pests, etc. Specific storage conditions can be monitored by the use of temperature/time records, while the physical location can be observed for cleanliness and freedom from vermin and dirt. Schedules for cleaning and vermin control programs should be in place and monitored in the storage area. Products released for dispatch should be physically located away from the quarantine area. The use of status stickers such as HOLD, QUARANTINE, REJECT, and PASSED will prevent substandard products from being dispatched. Electronic records should clearly indicate the status of finished goods in the storage area, and provision must be made to ensure that only released products are available for distribution to customers. The design of food storage areas should take into account access to goods, personnel, and forklifts, ease of cleaning, drainage, lighting, and ventilation.

Refrigerated foods such as sous vide and other chilled foods have gained popularity worldwide. These foods are more susceptible to mishandling than frozen or shelf-stable products and therefore need to be managed carefully. Training of personnel in the safe handling of food during transport, monitoring temperature/time records in refrigerated trucks, maintaining cleanliness and hygiene, and correct delivery procedure are some of the controls that can be exercised to reduce or eliminate the hazards due to transport and storage. Routine inspection and audits can be used to monitor the effectiveness of the storage, dispatch, and distribution system.

57.7.7.1.5 Premises

Control methods that can be used to prevent the occurrence of hazards and for safe operation within the premises of the food production area depend upon the proper design and layout of processing areas. Several control measures can be employed to prevent potential hazards: (i) pest control program in the plant, (ii) a scheduled maintenance program, (iii) regular inspection of vents and overhead pipes, (iv) use of guarded light fittings, (v) filtered air supply in the processing area, (vi) temperature/time records in manufacturing and storage areas, (vii) regular cleaning program for walls, floors, and ceiling, (viii) monitoring the quality of water supply and its temperature when used for sterilizing/ sanitizing of equipment, and (ix) waste disposal program to prevent the entry of rodents and pests. Health and safety regulations also provide some measures of controls over the hazards that originate in a food-processing facility. Regular audits should be carried out to ensure that health and safety regulations are observed.

57.7.7.1.6 Personnel

An HACCP program should take into account the hazards due to poor handling of food in a production facility and at food serving stations. In catering facilities and chilled and frozen food premises, poor physical health and lack of personal hygiene of staff represent a major risk [25]. Food handlers can be a major source of pathogenic bacteria. Incidences have been noted where personal articles such as pens, paper, jewelry, metal items, cigarette butts, and chewing gum have been incorporated into food products. Entry of these hazards can be controlled by introducing a policy covering prohibition of smoking, chewing gum, and wearing jewelry, maintaining personal hygiene, use of clean uniforms, monitoring illnesses, and regular medical care, and regular audits to monitor the effectiveness of documented procedures for handling food. All garments should be clean and free of soil. Freshly laundered garments should be provided for food handlers on a daily basis. Wherever appropriate head covers should be worn. Besides being unpleasant, hair is also a source of microorganisms [26]. Workers who handle food should not have cuts or infectious diseases. Such workers should be prevented from handling food. Touching prepared food with bare hands should be avoided. Suitable hand washing and drying facilities should be provided near work stations.

57.7.7.2 Measures at Postprocessing and Packaging Stages

Contamination of food products can also occur at postprocessing and packaging stages. Food manufacturers and retailers should be aware of the need to handle food in a safe and hygienic manner. Manufacturers and retailers have the responsibility to ensure that food products are not abused by the consumer after purchase.

57.7.7.2.1 Retail

Before food reaches the consumer, the retailer is responsible for maintaining the safety of all food products in his/her care. The retailer must store the food at the recommended temperature, and adequate care has to be taken when food is handled. The control measures therefore relate to monitoring temperature/time records during storage, inspection of equipment and facilities, auditing, training of staff, and use of tamper-proof and tamper-evident packaging.

57.7.7.2.2 Food Service

Food is presented to the consumer in a variety of ways. Some foodservice systems are prone to microbiological and other hazards. The techniques of HACCP that can be applied to food-production systems are also valid in food-service systems. The presence in food of certain microorganisms or their metabolic products in amounts sufficiently large to cause illness when consumed is a major concern. The population is at greater risk in this respect from the food-service establishments [27]. Hazards that need to be controlled are linked with several factors, including the composition of menus and individual food items, particularly raw materials not subjected to further processing, storage, preparation, handling, and holding procedures. The control methods include (i) selection of suppliers, (ii) inspection of raw materials on arrival, (iii) temperature/time control in storage and food-handling areas, (iv) monitoring personnel hygiene and food-handling practices, (v) sanitation of utensils and handling equipment, (vi) provision of adequate covers to protect from insects, and (vii) control of entry of insects

57.7.7.3 The Consumer

57.7.7.3.1 Food Preparation

Outbreaks of food poisoning due to poor handling of food in the home are not uncommon. Food spoilage due to pathogenic microorganisms as well as hazards from foreign objects can occur during the preparation of food. Consumer awareness of the potential hazards of handling food in the household is important in order to ensure the safety of foods prepared at home. The hazards can be controlled by checking containers prior to purchase, handling the product correctly on the way home, properly storing ingredients and food, keeping kitchen equipment clean, preparing food correctly, and managing the pantry appropriately [28].

57.7.7.3.2 Food Usage

Prepared food products such as ham, cheese, and sauces may be consumed directly or incorporated into other foods. Hazards can occur due to consumer abuse. The manner of reconstitution of the food and the consumer groups such as diabetics for whom the food is made should be indicated on the label. Limited control methods are possible in the hands of the consumer. Controls can be exercised through the provision of consumer information as to how the food product should be handled, used, and stored. Warning labels such as use-by dates and storage conditions, use of temperature/time indicators on sensitive and high-risk food items, and packaging design that minimizes abuse by the consumer are also ways in which the food processor can help the consumer minimize hazards.

57.7.8 Hazard Analysis

Hazard analysis is the first principle in the implementation of the HACCP program, and it is necessary to identify those hazards which must be eliminated or reduced to an acceptable level in order to produce safe food. A hazard analysis serves three purposes: (i) to identify the hazards of significance to food safety, (ii) to select critical hazards on the basis of risk to the consumer, (iii) to identify potential hazards that warrant specific preventive measures [29]. When the same product is manufactured by different food-processing organizations, the hazards will depend upon the following: (i) sources of ingredients, (ii) product formulations, (iii) processing machinery and equipment, (iv) processing and preparation procedures, (v) duration of processes, (v) storage conditions, and (vi) experience, knowledge, and attitudes of personnel [18].

All existing and new products must be subjected to hazard analysis, and when raw materials, product formulations, processing or preparation procedures, packaging components, distribution, and/or the use of product changes, the original hazard analysis has to be reviewed. A systematic approach to hazard analysis involves the assessment of biological, chemical, and physical hazards in all facets of the food production operation including delivery to consumers. Biological hazards occur when there is potential for harmful bacteria to contaminate the food. Chemical hazards exist when food is contaminated with substances such as pesticide residues, toxic metals, cleaning agents, and sometimes food additives and preservatives in excessive amounts. Physical hazards can exist when particles such as glass fragments, metal pieces, wood, hair, jewelry, or dirt contaminate the food. A suitable form for hazard analysis is shown in Table 57.10 [30]. The analysis should cover all the steps from receiving raw materials to delivery to consumers as given in the flow diagram.

TABLE 57.10
Hazard Analysis Form

Processing Step	Hazard Type	Significant? (Y/N)	Hazard Description	Control Method
	Biological			
	Chemical			
	Physical			

57.7.9 Assessing the Hazard Potential

An important step in implementing a HACCP program is the assessment of potential hazards. Hazard analysis requires knowledge of pathogenic organisms or any agent that could cause spoilage of the product and be harmful to consumers. A broad understanding of how these hazards could arise is also essential for a complete assessment. An assessment of potential hazards involves a detailed examination of the following: Raw materials, process, product, and end-use. These are assessed on the basis of biological, chemical, and physical hazards. Different systems have been used to assess the hazards associated with food products [31, 32]. Suitable layouts for the assessment of the hazards are described in the sections that follow.

57.7.9.1 Assessment of Raw Materials

The hazards related to raw materials can be grouped under microbial, foreign matter, and those associated with transportation and storage.

57.7.9.1.1 Biological Hazards

Some food products are more prone to microbial contamination that others, e.g., food products such as fish and meat are more likely to be contaminated with microorganisms than are fruits and vegetables. Chlorinated water and food ingredients such as salt generally do not carry microorganisms. Several factors are important for the growth of microorganisms. These factors are discussed in other chapters and must be taken into consideration in assessing the risk potential. Microbial growth may also occur during uncontrolled transport and storage conditions. Products such as salt and sugar do not require special storage conditions, whereas maintaining a prescribed temperature/time is important for chilled food.

57.7.9.1.2 Physical Hazards

Common types of foreign matter that are of concern in a food-production environment are soil, metal objects, personal

articles, etc., and the possibility of such contamination should also be assessed. Some food products may be damaged or undergo deterioration during transport. The extent of damage or deterioration that can occur under uncontrolled conditions of transport should also be considered when the raw materials are assessed.

57.7.9.1.3 Chemical Hazards

The raw materials may also be contaminated with chemicals and other pesticide residues. The possibility of such contamination should be considered during the assessment of raw materials.

57.7.9.1.4 Assessing the Risk

Risk can be high or low. For example, cooked food such as fish, meat, and eggs has a low risk in contrast to uncooked food. Even if the hazard is eliminated at a later stage of the process, any risk associated with raw materials should not be ignored.

57.7.9.2 Assessment of the Process

The assessment of the process involves analysis of each step. At each step of the process, consideration has to be given to the possibility of contamination with biological, physical, or chemical hazards.

57.7.9.2.1 Biological Hazards

Microbial destruction is a critical factor in food processing, and if adequate controls are not in place, microbial contamination is a distinct possibility. During the handling of food items, microbial contamination can occur. However, microbial growth will take place only if the material is a suitable substrate and is stored at a condition suitable for growth.

57.7.9.2.2 Physical Hazards

Processes such as sieving, washing, inspection, and metal detection are designed to remove or reduce foreign matter present in a food product. The assessment is based on the efficacy of the process. New foreign matter can be introduced during certain steps in the process. For example, plastic pieces can become embedded in the food product during a packaging operation, and metal pieces or shavings can get into the product via faulty machinery.

57.7.9.2.3 Chemical Hazards

Any equipment that comes into direct contact with food items can contaminate the food if it has not been thoroughly cleaned. On the other hand, carton closure equipment that does not come into contact with food directly cannot be considered a possible hazard source.

57.7.9.2.4 Control Measures

If the hazard is not eliminated downstream, adequate control measures should be exercised at the step under consideration. If vegetables are washed for the purpose of removing foreign matter and intended to be consumed uncooked, the washing step is critical and needs a high degree of control because of possible microbial contamination. However, if the vegetables are intended to be cooked, the washing step is for the purpose of removing soil and dirt and therefore needs a low degree of control. Displaying raw chilled seafood, poultry, and meat needs a high degree of control because it is important to keep the display temperature below 4°C in order to limit bacterial growth [33]. The consequences of failing to do so can be serious. Process steps such as filling trays and handling of food require a moderate degree of control.

57.7.9.3 Assessment of the Product during Storage and Delivery

The methods by which food should be stored and delivered depend on the characteristics of the food product. The product is evaluated on the basis of hazards associated with its stability. Consideration has to be given to storage conditions, packaging requirements, and delivery instructions necessary to prevent the product from undergoing deterioration or spoilage.

57.7.9.3.1 Biological Hazards

Products such as bottled wine, canned foods, and jam do not require special storage conditions, and hence there are no hazards associated with storage. For perishable foods such as meat, fish, and ice cream, the storage conditions are critical to prevent the growth of microorganisms. Vegetables require special storage conditions, but if abused, the hazards that can occur are less critical. Chilled and frozen foods may be subjected to unfavorable storage conditions (temperature/time) over long periods during transport. These food products require special packaging, and the hazards associated with such products are critical.

57.7.9.3.2 Physical Hazards

Food is transported over long distances and is subject to a wide variety of storage conditions and handling techniques. The hazards that can occur during the transport of food substances are assessed as to the extent of damage or deterioration. Few or no hazards are associated with food products that do not require special storage conditions or handling techniques. Some food products packed in glass or plastic containers require special storage conditions during transport to prevent damage and subsequent spoilage.

57.7.9.3.4 Chemical Hazards

Chemical hazards in a food product during storage and delivery are often caused by unclean containers. Food ingredients are also transported to food producers, and in some instances, contaminated substances may be transported in the same vehicle [34].

57.7.9.3.5 Control Measures

Controls are not necessary for food products such as canned foods, sugar, and salt. When chilled and frozen food products are transported, stringent control measures should be exercised. Some products that are transported over short distances in insulated packaging may not require special storage

conditions, and failure to maintain control over temperature/time may not have serious consequences.

57.7.9.4 Assessment of End Use

During the last few years, food manufacturers have been aware of the increase in the cases of consumer dissatisfaction and complaints about food products [28]. Mishandling of products that leads to deterioration of quality has been cited as one of the causes of consumer dissatisfaction. Hazards can sometimes occur in the hands of the consumer as a result of inappropriate usage and abuse by the consumer.

57.7.9.4.1 Inappropriate Usage

Food products that can be safely consumed by the general population cause no risk. Some food products that can be safely consumed by a section of the population may not be tolerated by others, although the effects of such a hazard may not be significant. The hazards associated with the usage of such foods are classified as low. The hazards that can occur, for example, as a result of incorrect labeling or wrong formulation of food products made especially for certain groups of people, such as the elderly, children with allergies, and diabetic patients, are critical.

57.7.9.4.2 Abuse by the Consumer

Food products that do not require special storage conditions or handling cause no hazards in the hands of the consumer. However, some food products have a low risk of being abused and require moderate care in handling, for example, bread left outside and open to air soon develops mold. Some foods such as cooked meat require special handling by the consumer to prevent spoilage that may not be obvious, and the consumption of such food may cause serious illness (e.g., *Salmonella* poisoning). These food products have a high risk of abuse.

57.7.9.4.3 Control Measures

Controls are not necessary for food products that cannot be abused. Foods that have a low risk of abuse require a moderate degree of control, and other food products that require special handling need strict control measures.

57.7.10 Critical Control Points (CCPs)

The determination of CCPs is the second principle of HACCP. This step follows the hazard analysis which identifies the potential hazards that can threaten the food supply and production. A critical control point in a food-processing system leads to an unacceptable health risk and can be effectively controlled to prevent or eliminate health hazards to an acceptable level of low risk.

57.7.10.1 Classification

Critical control points can be generally classified as CCP1 and CCP2 [35]. CCP1 is defined as a step or location in a food-processing system that on its own effectively eliminates a hazard, e.g., metal detection in food products and sterilization. CCP2 is defined as a step or location in a food-processing system that contributes to the control of a hazard but does not guarantee elimination, e.g., inspection and pasteurization.

It is important to distinguish between CCPs and control points that are less critical in ensuring food safety. Several key points to note in determining the CCPs are (i) CCPs should not be restricted to a minimum or maximum number, (ii) a CCP is specific to a product and process, (iii) CCPs should not be duplicated, (iv) CCPs should only be introduced when it is necessary to eliminate or reduce a health hazard, (v) CCPs should always be developed by consulting an expert when there is doubt about a product or a process, and (vi) development of CCPs requires the use of common sense [15].

The presence of a control downstream should not be considered a reason to neglect controls in proceeding steps. For example, wines are tested for pesticide residues prior to bottling, and even then the grape grower has the responsibility to control the spray program. Any opportunity to eliminate or minimize the occurrence of a hazard should not be overlooked.

57.7.10.2 Location of CCPs

HACCP techniques enable the food processor to identify hazards and risks, focus on where they pose a threat to the safety of food, and develop the means to control them. The actual location of a CCP depends upon the type of hazard, the ingredients, packaging, processing procedures, storage, and handling. Emphasis should be placed on the prevention of entry rather than detection after they have been introduced. CCPs should be introduced as early as possible in the food-processing system and close to the origin of the hazard. All precautions must be taken to prevent the entry of new ones [35]. Hazards associated with raw materials should be controlled at the source, i.e., the supplier. This minimizes the risk of entry of hazards and avoids unnecessary inspection of raw ingredients on receipt. Therefore, preprocessing techniques such as washing and sorting will be more effective in controlling hazards.

Food-production processes are often associated with more than one CCP. For example, in the production of entrees in conventional, cook/chill, and cook/freeze food service systems, time–temperature is a CCP throughout production in each of the models. Equipment and personal sanitation are also CCPs that should be regularly monitored using standards and criteria established by the food-processing system [36]. The inspection of the finished product usually verifies the effectiveness of controls placed so far.

57.7.10.3 Determination of CCPs

True CCPs have often been confused with control points, and as a result, a large number of CCPs are identified, making the HACCP system unworkable. For example, in a commercial process of smoked fish, it is possible to identify many individual steps, but only three can be considered critical: Salt penetration, smoking, and storage [21]. There are two approaches for identifying the CCPs: Decision tree developed by the Codex Alimentarius Committee of Food Hygiene [37, 38] and risk analysis.

57.7.11 Identification of CCPs

57.7.11.1 Decision Tree

The decision tree [30] employs a systematic approach and a logical process at every process step for every hazard identified as significant in the food production operation. It promotes further discussion by the HACCP team and hence improves the quality of the outcome. The principal elements of the decision tree are shown in Table 57.11 as a series of questions.

Q1. Do control measures exist at this or subsequent steps for the identified hazard?

This question refers to the use of control measures such as temperature control, visual inspection, or use of metal detectors at this step or subsequent steps in the food-processing operation to control the hazard. If there are no control measures, the team should indicate how the hazard will be controlled before or after the manufacturing process (outside the control of the organization). For example, pesticide residue in grapes is controlled by the grape grower. Alternatively, the operation, process, or product could be modified to ensure that a control measure exists.

Q2. Does this step eliminate or reduce the likely occurrence of the identified hazard to an acceptable level?

For each operation, the team should define the acceptable levels. Examples of controls that are designed to eliminate or reduce the likely occurrence of a hazard include chlorination of water, pasteurization, use of metal detectors, and cleaning procedures, etc. Question 2 applies to process operations, and it is not applicable to incoming raw materials.

Q3. Could contamination with the identified hazard occur in excess of acceptable levels or increase to unacceptable levels?

This question refers to the likelihood of the hazard having an impact on the safety of the product. The response to this question should be based on the risks, the company's complaint reports, and scientific literature on the subject. If the contamination is not likely to represent a threat to human life, the response to this question is "no."

Q4. Will a subsequent step eliminate the identified hazard or reduce likely occurrence to an acceptable level?

Question 4 is designed to identify those hazards that are known to represent a threat to human health or that could increase to an acceptable level, and that will be controlled by a downstream operation. If the response to this question is a "yes," it is necessary to identify the subsequent step that controls the hazard, thus proceeding to the next identified hazard. The questions given in the decision tree should be asked for each significant hazard at each process operation, including receipt and handling of raw materials. All CCPs identified by the team must be implemented and cannot be replaced by other controls elsewhere in the operation.

57.7.11.2 Risk Analysis

Food safety is a significant public health issue worldwide with major costs to the health authorities. Uncontrolled application of agricultural chemicals, environmental pollution, use of non-permissible additives, and other abuses of food along the food chain can potentially introduce or fail to reduce hazards related to food production, delivery, and service. Therefore, food safety programs designed to protect consumers have to meet several challenges: (i) the emergence of new pathogens and other hazards, (ii) the re-emergence of pathogens and other hazards, and (iii) the threat of bioterrorism with increased awareness of food additives and food safety among consumers, analysis of risks associated with food become extremely important. The level of risk to the consumer depends on the degree of control exercised by growers, suppliers, food processors, and food regulatory authorities to eliminate or minimize the risks to acceptable safe levels. Risk analysis is an evolving discipline, and the methods used for the assessment and management of risks are still being developed [39].

It is important to understand the difference between hazard and risk. A hazard is a biological, chemical, or physical agent in, or condition of food that may have an adverse health effect. In contrast, risk is a function of the probability of an adverse effect

TABLE 57.11
CCP Decision Tree

Question Number	Question	Response "Yes"	Response "No"
1	Do control measures exist at this or subsequent steps for the identified hazard?	Proceed to Q2	If the control is necessary modify the process
2	Is this step specifically designed to eliminate or reduce the likely occurrence of the identified hazard to an acceptable level?	CCP	Proceed to Q3
3	Could contamination with the identified hazard occur in excess of acceptable levels or increase to an unacceptable level?	Proceed to Q4	Not a CCP
4	Will a subsequent step eliminate the identified hazard or reduce likely occurrence to an acceptable level?	Not a CCP	CCP

and its severity of impact on the affected population [40]. The risk analysis process involves three distinct steps: Risk assessment, risk management, and risk communication. The purpose of risk assessment is to identify the hazards and their immediate, interim, and long-term effects on human health. Risk management establishes appropriate controls to eliminate or reduce these risks. The aim of risk communication is to develop methods to communicate this information to consumers.

57.7.11.2.1 Risk Assessment Risk assessment consists of four components.

1. Hazard identification
2. Hazard characterization—quantitative and/or qualitative evaluation of the adverse effects of the hazard on humans
3. Exposure assessment—quantitative or qualitative evaluation of the likely degree of exposure to the hazard
4. Risk characterization—integration of the first three components to arrive at an estimate of the likely adverse effects in the target population

Once the hazards have been identified, they should be assessed on the basis of established data. Each hazard can be assigned a numerical risk level based on the severity of outcome and the likelihood of occurrence of the hazard [41]. Severity is classified at three levels:

High severity (score = 3)

Microbial contamination causing life-threatening illness, e.g. *Clostridium botulinum*, *Salmonella typhi*, *Vibro cholerae*, or contamination with a chemical or foreign object causing life-threatening or permanent illness or injury.

Medium severity (score = 2)

Microbial contamination causing chronic illness, e.g. *Campylobacter*, *Vibrio parahaemolyticus*, or contamination with a chemical or foreign body causing temporary illness or injury.

Low severity (score = 1)

Microbiological contamination causing moderate illness, e.g. *Listeria monocytogenes*, *Staphylococcus aureus*, or contamination of a chemical or foreign body causing discomfort, nausea, etc. [23]. The likelihood can also be categorized as high (score = 3), medium (score = 2), and low (score = 1). High likelihood indicates that the hazard will occur; medium likelihood indicates that there is a reasonable chance of occurrence; and low likelihood denotes that the occurrence will be rare. Risk level = likelihood × severity. Risk level = 1–3: Low risk—establish control measures where appropriate. Risk level = 4–6: Medium risk—establish control measures. Risk level = 7–9: High risk—CCP.

In the canning process, the likelihood of *Clostridium botulinum* surviving retorting is very low (1) but the severity of the illness (botulism) is high (3). Thus, the risk level = 3 and the retorting is a control point and not a CCP. A better approach for risk assessment takes into consideration the severity of the illness as well as customer complaint reports and product recalls [42]. The ratings for severity are as follows: (i) fatality, (ii) serious illness, (iii) product recall, (iv) customer complaint, and (v) not significant. Likelihood ratings for each food hazard are: A, common repeating occurrence. B, known to occur or "it has happened" (own information). C, could occur or "I have heard it happening" (published data). D, not expected to occur. E, practically impossible. Based on the above ratings, significant factors are assigned to various combinations as shown in the matrix in Table 57.12.

Example of an assessment of significance:

> Process step: Receiving salmon for the production of vacuum-packed hot smoked salmon. Potential hazard: Pathogens, parasites in fish. Severity: 2 (serious illness). Frequency: D (not expected to occur in finished product because of subsequent steps).
>
> Significance: 12 (not a CCP). Control measures: Received frozen, supplier HACCP compliant with specifications, control smoking/cooking at subsequent step.

57.7.11.2.2 Risk Management

Risk management is the process of implementing policy alternatives compatible with the results of risk assessment and applying appropriate control options, including regulatory measures. The aims of the risk management program are to (i) establish the significance of estimated risk, (ii) compare the costs of reducing this risk to the benefits gained, (iii) compare the estimated risk to benefits to the consumer, and (iv) promote regulatory and other changes necessary to reduce the risk.

TABLE 57.12
Matrix for the Assessment of Significance of Risk

Likelihood →		A	B	C	D	E
Severity	1	1	2	4	5	11
↓	2	3	5	8	12	16
	3	6	9	13	17	16
	4	10	14	18	21	23
	5	15	19	22	24	25

Note: Shaded areas = CCPs

The outcome of the risk management program is the development of standards, codes of practice, and other guidelines for food safety. Risk management decisions are based on the outcome of the risk assessment process, food processing, quality and food safety requirements, food handling and distribution requirements, and food quality and safety standards to control hazards in food production. These decisions are implemented, and their effectiveness of control measures is monitored to ensure that the food safety objectives are met.

57.7.11.2.3 Risk Communication

Risk communication is the final element of the risk analysis process. According to the Codex Alimentarius definition, risk communication is an interactive process of exchange of information and opinion on risk among risk assessors, risk managers, and other interested parties. The responsibility for food safety rests with all involved at all stages of the food chain, including consumers. They should be provided with appropriate information on potential hazards and precautions to be taken to eliminate or reduce the risks. Consumers also need to be aware of the control measures exercised by the regulatory bodies to ensure food safety.

The purpose of risk communication is to educate the general public and specific target groups such as the elderly, diabetics, etc., on food hazards and their risks to general health and wellbeing. Communication provides information necessary to prevent, minimize, or reduce risks associated with food intake to acceptable, safe levels through voluntary or regulatory control mechanisms. This information also provides consumers with the power to exercise their own control measures to safeguard their health.

57.7.12 Establishing Critical Limits for Each CCP

Establishing measurable limits (specifications) for each CCP is the third principle of HACCP. The critical limits define the criteria for acceptability or rejection and thus are the operational boundaries for each CCP. These limits may be derived through various sources: Experiments through validation, regulatory requirements, codes of practice, or other valid sources. Critical limits should be established for the following food production operations as applicable: (i) distribution processes, (ii) receiving operations, (iii) storage, (iv) thawing, (v) production, (vi) hot holding, (vii) cooling, (viii) processing, (ix) reheating, (x) cold holding, (xi) transporting, (xii) recipe flowcharting, and (xiii) employee training.

The measurable limits are often defined by time, temperature, physical attributes, acidity, pH, moisture content, water activity, salt concentration, and chlorine levels. Purchasing management procedures should include clearly defined specifications, and the control limits may refer to a successful outcome of an audit at the supplier's premises. The person who receives goods must ensure that the food is received in good condition, free from spoilage and tampering, and that it meets the organization's in-house specifications. It is essential that the temperature of perishable products and the cleanliness of the vehicle/container are checked on receipt. Training requirements have to be established for all employees who handle food. These may include proper handwashing techniques, use of clean uniforms and hair covers, communicating company policies on smoking in the workplace, wearing jewelry, etc. Cross-contamination is an important hazard that is sometimes overlooked. Raw and cooked products should be physically segregated in storage. Critical limits for cross-contamination may include storage conditions and proper use of cutting boards. Table 57.13 shows some examples of critical limits [18].

57.7.13 Establishing Procedures and/or Equipment for Monitoring CCPs

Monitoring is defined as planned measurement or observation to ensure that the CCP is under control and is the fourth principle of HACCP. Monitoring procedures should be carefully defined such that loss of control can be detected. It is essential that responsibility is assigned for observation or testing. The test results have to be accurately recorded for future reference. The purpose of monitoring includes the following: (i) to evaluate the effectiveness of the system's operation at the CCP, (ii) to determine instances of deviation from a critical limit, (iii) to provide evidence that the performance level of the operation at the CCP complies with the HACCP plan.

Monitoring can be performed at defined time intervals or continuously. The latter is more reliable and shows deviations from the specified limits, allowing timely corrective action to be taken. When monitoring is done at time intervals, the

TABLE 57.13
Some Examples of Critical Limits

Hazard	CCP	Control Limit
Bacterial pathogens (non-sporulating)	Pasteurization	72°C for at least 15 seconds
Metal fragments	Metal detection	Metal fragments larger than 0.5 mm
Bacterial pathogens	Drying over	A_w less than 0.85 for controlling growth in dried food products
	Acidification step	Maximum pH of 4.6 to control *Clostridium bolulinum*
Excessive nitrite	Curing/brining	Maximum 200 ppm sodium nitrite in finished product
Food allergens	Labeling	Legible label containing a list of correct ingredients
Histamine	Receiving fish	Maximum 25 ppm histamine levels in evaluation of tuna for histamine

frequency of monitoring should provide confidence that the CCP is under control. When monitoring systems are designed it is necessary to consider the time lapse between measurement and the results. On-line measurements are rapid and provide results immediately. On the other hand, microbiological tests provide results after a few days, and therefore, finished products have to be held under quarantine until the test results are known. All monitoring equipment should be subjected to regular calibrations. There are two types of monitoring: Measurement monitoring and observation monitoring [43].

Observation monitoring usually involves the use of checklists and includes visual checks (sight, smell, and taste), visual observations for some physical characteristic (presence of foreign objects), and checks for hygiene and cleanliness. Observation monitoring is generally easy to implement, but there are some disadvantages. Results of observation monitoring often require interpretation, and the operators must be sufficiently trained to make sound judgment.

Measurement monitoring often involves instrumentation and can be automated. The results are straightforward and do not require subjective judgment. Measurement techniques can be designed such that the findings can be easily interpreted. The results will also demonstrate trends and highlight subtle changes.

57.7.14 Establishing Corrective Actions

Establishing a corrective action procedure is the fifth principle of the HACCP plan. Corrective action is necessary when a deviation at a CCP has exceeded the critical limits. A deviation is critical when it results in an unacceptable consumer health risk, which must be resolved promptly. Deviation procedures are a predetermined and documented set of actions to be accomplished when a deviation occurs. Corrective actions enable the cause of the non-compliance to be corrected and the non-compliant product to be managed. Product control includes proper identification, segregation, and disposition of the affected product.

After the appropriate corrective action is taken, it may be necessary to review the HACCP plan. The corrective action procedures should include an investigation to determine the cause of the problem, effective procedures to prevent a recurrence, and verification of the effectiveness of corrective action.

57.7.15 Verification Procedures

The sixth principle of HACCP is the verification of operational procedures. It is the task of application of methods, procedures, tests, and other evaluations, in addition to monitoring to determine compliance with the HACCP plan [18]. The purpose of verification procedures is to assess the effectiveness of the plan and ensure that the HACCP system is compatible with the plan. Although it is carried out at planned intervals on completion of the study, verification may also have to be performed when there is a change in the ingredients, product, or process or when a deviation occurs or a new hazard is identified. Some of the verification activities are (i) audits covering all operations of food production, (ii) reviews of menu and recipes and confirmation that documented methods are followed, (iii) maintenance and calibration checks, (iv) verification of flow charts, (v) cross-contamination possibilities, (vi) all records including relevant training records, (vii) corrective action reports, and (viii) compliance with regulatory requirements

57.7.16 Documentation and Record-Keeping

Record keeping is the last principle of the HACCP plan and is an essential requirement of the HACCP program. Records demonstrate the history of the process, its measurements, deviations at the CCP, and corrective actions taken. Record keeping is also an essential regulatory requirement. Regulations governing food production dictate the type of records to be maintained by the food processor. For example, according to FDA's HACCP regulations, seafood operations are expected to document and follow basic sanitation standards [30]. An HACCP manual includes, but is not limited to, the following documents: (i) profile of the organizations, (ii) policies, e.g., hygiene, cleanliness, smoking, etc., (iii) products manufactured by the organization, (iv) prerequisite program referenced, (v) flow diagrams, (vi) hazard analysis, (vii) HACCP schedule, (viii) consumer complaint procedure, (ix) recall procedure, (x) corrective action and disposal of nonconforming goods procedure, and (xi) recording and documenting control procedure [44].

Records and documents may be in any form, e.g., charts, written procedures, electronic records. The records have to be regularly reviewed to ensure that HACCP controls are effective, correct data are being recorded at specified intervals, and that operators are completing their tasks as instructed. Most of the records associated with the HACCP program are associated with monitoring and corrective actions. They can be simple check sheets or complex control charts. The type of records to be maintained depends on the nature of the food-processing operations. Monitoring the temperature is essential for processes that employ refrigerators, freezers, dishwashers, steam equipment, hot and cold cabinets, and ovens. Some examples of records to be maintained are (i) pest control records, (ii) calibration records, (iii) maintenance logs, (iv) shipping records, (v) premises inspection reports, (vi) release reports, (vii) training records, (vii) calibration reports, (ix) consumer complaint and corrective action reports, and (x) control charts as applicable. Record-keeping should not be treated as mere paperwork. It is the key system to manage, verify, and validate the HACCP program [45].

57.7.17 Validation Procedures

While verification can be performed by audits and other methods at scheduled intervals and as required, the effectiveness of operational processes and methods can be determined only by a proper validation. Validation is the process of ensuring that the procedures are effective [46]. A process can be verified as correct but may not be valid to achieve the

desired result. For example, the process of cooling a stockpot of soup in a refrigerator may comply with documented procedures (verification), but the long time required for the temperature to drop in the center of a large container can permit bacteria to continue to grow (validation). Hence, the validity of the cooling process (refrigerator in contrast to blast chiller) is in doubt. Often equipment or procedures are unique to the organization, and therefore the operating characteristics of the process will become important in the validation of a critical limit. Food science is an evolving discipline, and it is essential that up-to-date scientific references are used in the validation process. Validation is an on-going planned procedure. However, the following factors may cause a review of the validation procedure: (i) when raw materials, product, or processes change, (ii) results of adverse audit reports, (iii) frequent deviation from the specified critical limits, (iv) new scientific data on potential hazards or processes, (v) consumer complaints, and (vi) product recalls.

57.8 CONSUMER PROTECTION

The primary objective of a HACCP system is to protect the consumer from harm caused by hazards associated with food products. A survey carried out by a task force has revealed four causes of consumer dissatisfaction with food products [28]: Unfamiliarity and expectations, price, defects, and mishandling by the consumer.

Food producers have a responsibility to ensure that all food products are adequately labeled. Information such as product description (particularly for new products), ingredients, shelf life, storage conditions, and special preparations, if any, should be provided to create awareness of the nature of the product and to prevent mishandling. Retailers as well as manufacturers can go a step further by providing information in the form of leaflets that explain handling of food products on the way home, storing of ingredients and prepared foods, and the potential hazards involved with handling foods in the household. Food producers, as well as retailers, have an important role to play in educating the consumer as to food safety.

57.9 MANAGEMENT OF THE HACCP PROGRAM

57.9.1 Review, Audit, and Recall Processes

57.9.1.1 Review

The HACCP program, just like the quality management system, is dynamic. With advances in food technology, food producers are constantly looking at new, rapid, and safe ways of processing food. The HACCP program should be flexible enough to adapt itself to changing circumstances. New knowledge gained about microorganisms also presents a challenge to already developed and implemented HACCP programs. An HACCP program should also take into account the variability and adaptability of agents responsible for hazards to human health.

It is easy for organizations that have a quality management system in place to incorporate a HACCP program into the already existing system. Procedures such as management responsibility, management review, and document approval and issue can be applied to the HACCP system. The function of the management representative then is to maintain the system through regular audits and reviews. The management representative responsible for the program should ensure that all new and current product specifications, results of audits, customer feedback, process conformance and product conformity, the status of preventive and corrective actions, actions taken since the last meeting, standards of practice, changes to procedures and equipment, engineering and microbiological data, safety controls, and monitoring systems are reviewed regularly by the HACCP team. It is the responsibility of the team to determine, in relation to current practices or new procedures, (i) the potential hazards in ingredients, products, and risks, (ii) whether the hazard can be eliminated or minimized, (iii) the effectiveness of a terminal heat treatment, (iv) the possibility of recontamination, and (v) the hazards associated with handling, storage, distribution, and product usage.

A hazard identification form (Figure 57.3) can be used to report the results found by the team. All accidents, misuse of ingredients, unsafe environment, and safety issues must be recorded and reported to the appropriate authorities. Authority must also be given to the operators to stop the process if in their opinion it is unsafe to operate. All safety issues must be dealt with immediately, and timely action must be taken to eliminate unsafe practices and equipment.

57.9.1.2 Audit

An HACCP audit can be defined as a systematic and independent examination to determine whether (i) HACCP activities and related results comply with planned arrangements, (ii) these arrangements are implemented effectively, and (iii) the arrangements are suitable to achieve the objectives. A schedule of audits must be prepared and carried out as planned. There is no international standard yet for the HACCP system equivalent to the ISO 9000 standard series. However, the HACCP system can be audited against the specified requirements of the system. To some extent, the British Retail Consortium (BRC) standard for food meets the requirements of a food safety standard [11]. It integrates the relevant section of the ISO 9000 standard, Codex Alimentarius, and GMP. The BRC standard has the following sections: (i) HACCP system, (ii) quality management system, (iii) general documentation requirements, (iv) factory environment standards, (v) product control, (vi) process control, and (vii) personnel.

HACCP audits should provide (i) an assessment of the adequacy of the existing system (ii) a benchmark against which improvements can be made and evaluated, (iii) evidence that contractual and legal requirements have been met, and (iv) feedback on safety issues. HACCP audits are carried out in a manner similar to quality system audits and typically apply to, but are not limited to, records and activities associated with control points and critical control points, training, and reviews. All non-compliances must be dealt with at the earliest

HAZARD IDENTIFICATION FORM

A. HAZARD

Identified hazard (include details such as product name, code, pack size etc.)
.....
.....
.....
.....
Identified by.....Date.....Time.....
Location.....

Potential effect on
health/safety.....
.....
.....
Severity score.....Likelihood score.....Risk factor.....

B. ANALYSIS

Contributing factors.....
.....
.....
.....

C. SOLUTION

Recommended method for reduction/elimination of hazard.....
.....
.....
.....

D. IMPLEMENTATION

Responsibility.....By when.....

E. MONITORING

Results of audit.....
.....
.....

FIGURE 57.3 Hazard identification form.

opportunity, and products related to these non-compliances should be kept under quarantine for thorough investigation.

57.9.1.3 The Product Recall Process

A reliable and well-tested method of recall should be in place to deal with a food item that has been established to be contaminated with a harmful ingredient or pathogenic organisms. Government regulations place a legal responsibility on food producers who recall food products for safety-related reasons to notify the authorities in writing within a specific period of initiating a recall (Figure 57.4). The traceability information will enable the affected product in the warehouse, in retail outlets, and in the hands of the consumer to be isolated. A suggested plan for a product recall is shown in Figure 57.5.

The text for the advertisement placed in the daily print media should comply with the statutory requirements and include (i) the name of the product and the producer, (ii) the pack size and a description of the packaging, (iii) any other details necessary for identification, (iii) the reason for recall, (iv) the necessity to identify and quarantine the stock, (v) the manner of disposal, (vi) if the hazard to the consumer is serious, indications of clinical symptoms and advice to consult a medical practitioner, and (vii) a toll-free telephone number to provide assistance to consumers. When the recall has been terminated, the recall team should review the effectiveness of the recall procedure and recommend changes,

OUR FACTORY LIMITED

PRODUCT RECALL FORM

REFERENCE: **NAME OF OUTLET:**
CONTACT PERSON: **DATE:**
TELEPHONE: **FAX:**

1. PLEASE REMOVE THE FOLLOWING PRODUCTS FROM SALE TO CUSTOMERS IMMEDIATELY

PRODUCT CODE SIZE
……
……
……
……

2. REASON FOR RECALL

……
……
……
……

3. PLEASE RETURN YOUR STOCK TO:

……

NOTES:
1. Please return the form to……
2. If you are not holding stock send a NIL return
3. All stock will be replaced at Our Factory expense

I have returned today……..(units)
To……..

……..
Manager Date:

Our phone: Our fax:

FIGURE 57.4 Product recall form.

if necessary. The recall team should document the following information: (i) the name of the product and pack size, (ii) the reason for recall, (iii) the cause of the problem, (iv) the chronological history of the recall events with actions taken, (v) the effectiveness of the recall, (vi) the total cost of the recall, (vii) the corrective actions taken, and (viii) the effectiveness of the recall.

In the case of a recall, the accuracy of information and speed with which action is taken is important. Although a mock recall may be impracticable, a regular traceability audit of a product should be carried out. This may include an audit of the ingredients used, process employed, laboratory findings, and the product distribution details. A sample recall notice is shown in Figure 57.6.

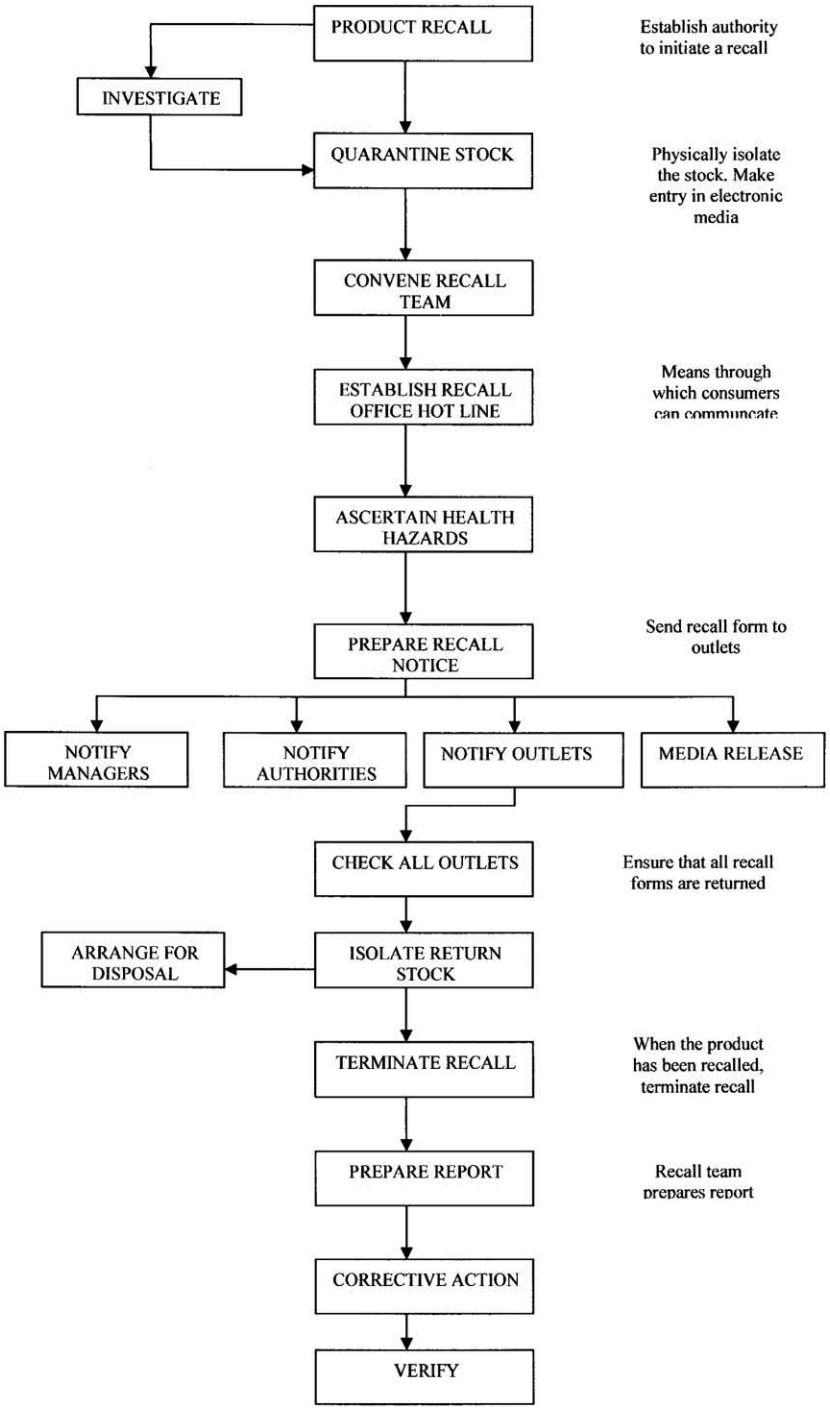

FIGURE 57.5 Recall plan.

57.10 BENEFITS OF IMPLEMENTING A HACCP SYSTEM

The HACCP system is a preventive and cost-effective approach to food safety and is more effective in preventing foodborne illnesses than traditional approaches. The application of an effective HACCP system has clear benefits for consumers, and industry as well as for regulatory bodies [47]. These benefits are summarized in Table 57.14.

57.11 HACCP IN THE OVERALL QUALITY SYSTEM

The survival of a business depends on its ability to satisfy the customer's needs and expectations at an affordable cost, which is achieved through an effective quality management system. It provides a basis for continuous improvement. The effectiveness of a quality management system can be assessed against standards such as ISO 9000. The quality management

FOOD RECALL NOTICE

FOOD PRODUCTS LIMITED STRAWBERRY JAM

FOOD PRODUCTS LIMITED IS RECALLING 375G JARS OF STRAWBERRY JAM, BECAUSE OF THE DISCOVERY OF GLASS IN TWO JARS OF FOOD PRODUCT'S STRAWBERRY JAM. THE RECALL APPLIES TO THE PRODUCT HAVING THE CODE L23A6.

THE PRODUCT HAVING THE ABOVE CODE SHOULD NOT BE CONSUMED.

AS A SAFETY MEASURE WE ARE RECALLING ALL SUPPLIES OF THIS PRODUCT WITH THE ABOVE IDENTIFICATION ON THE LABEL.

THERE HAVE BEEN NO REPORTS OF INJURY OR ILLNESS. HOWEVER, ANY PERSON CONCERNED ABOUT THEIR HEALTH AS A RESULT OF CONSUMING THE PRODUCT SHOULD SEEK MEDICAL ADVICE.

PLEASE RETURN THE PRODUCT TO THE POINT OF PURCHASE FOR A REFUND OR PHONE TOLL FREE……..

THE RECALL DOES NOT APPLY TO ANY OTHER FOOD PRODUCTS LIMITED STRAWBERRY JAM HAVING A DIFFERENT CODE OR ANY OTHER FOOD PRODUCTS LIMITED PRODUCT.

WE SINCERELY APOLOGISE FOR ANY INCONVENIENCE CAUSED BY THIS RECALL.

FOOD PRODUCTS LIMITED
(ADDRESS)
FAX

FIGURE 57.6 Recall notice.

system should be designed to meet the individual needs of the organization. Food manufacturers, while being aware of the quality of foods, should also realize that food safety is an absolute requirement. Regulatory authorities all over the world enforce laws aimed at protecting the consumer from harmful food. HACCP is a management tool that focuses attention on food safety and complements the quality management system. Implementation of a quality management system and a HACCP program both require teamwork. The requirements of a HACCP program are embodied in the seven principles as defined by the National Advisory Committee on Microbiological Criteria for Food [48].

The procedures relating to the HACCP system can be developed using the seven principles as the basis. An ISO 9000-based quality management system can easily be integrated with the HACCP program [44]. The clauses in the ISO 9000 standard such as responsibility, management review, and audit can be applied while keeping the focus on safety as well as quality. An HACCP system can be incorporated into the quality management system by making references to individual clauses such as product realization, corrective and preventive actions, internal audits, document control, and others common to both systems. The HACCP system should not be limited to the seven principles and should also include procedures relating to HACCP planning, customer complaints, and control of HACCP records, some of which are prerequisites.

TABLE 57.14
Benefits of Implementing the HACCP System

Benefits for Consumers
Lower risk of foodborne illnesses
Greater awareness of food safety
Greater consumer confidence in food supply
Better quality of life through health and socio-economic benefits
Benefits for Industry
Greater confidence in product
Minimize legal and insurance costs
Increase market access
Lower waste, fewer or no recalls, minimum or no reprocessing and corrective action
A consistent product
Enhanced staff commitment to food safety
Lower business risk
Benefits for Regulatory Bodies
Improved health among the community
More efficient food control
Lower public health costs
Trade promotion
Greater confidence of the community in the food supply

57.12 CASE STUDY (I): PRODUCTION OF CHICKEN AND VEGETABLE SALAD

57.12.1 Product Description

Chicken and vegetable salad is a ready-to-eat chilled product prepared with cooked chicken, freshly sliced celery,

and cooked, peeled, and diced potato in a dressing mixture. Chicken breasts (skinless, boneless) and other ingredients are bought at the market. The dressing mixture consists of a pasteurized mayonnaise base blended with spices. The product is put into plastic trays, which are covered with a peelable foil membrane and packed in a cardboard outer package. The product is refrigerated and brought to the refrigerated case as required.

57.12.2 Assessing the Hazard Potential of Chicken and Vegetable Salad

A flow diagram for the production of chicken and vegetable salad has already been described (Figure 57.1). The next step in the HACCP technique is to assess the hazards associated with the raw materials, processing steps, product, and end usage. It will then be possible to determine the raw materials and the processes that are critical. The whole process is then examined in order to establish the critical control points using the decision tree.

57.12.2.1 Raw Materials

Of the raw materials, the most hazardous item is chicken. It may be contaminated with pathogenic bacteria. The storage conditions after collection and during transport are also significant. Therefore, the cooking step is very critical. Potatoes may carry bacterial spores on the skin. If the potatoes are received washed, the washing step is only a control point. Celery may be contaminated with bacteria and spray residues. Although chicken, potato, celery, and other ingredients may be contaminated with foreign matter, washing and/or inspection prior to use will reduce the risk of contamination. Mayonnaise, being acidic, may be contaminated with acid-tolerant bacteria. Tables 57.15–57.18 show the hazards associated with the raw materials used in the production of chicken and vegetable salad.

57.12.2.2 Process

The process assessment of chicken and vegetable salad is given in Table 57.19. Potatoes are washed for the purpose of removing foreign matter from the skin. Cooking chicken in the only lethal step in the whole process and requires careful control of temperature and cooking time. Potatoes are also cooked, but the bacterial spores residing on the skin may not be destroyed. After cooking, there are several steps (peeling, trimming, and chopping) that involve manual handling. All such steps are hazardous, and there are no lethal steps downstream. Washing of celery may reduce the level of vegetative pathogens but will not eliminate them. Microbial contamination can occur during the mixing operations. The salad is hand-filled into trays and exposed to the environment. The frequent manual handling during the filling operation is a health hazard. The temperature of chilled storage and display cabinets must be controlled to prevent the growth of microorganisms. All steps recognized as significant should be considered for the identification of CCPs in the final analysis.

57.12.2.3 Product

Microbial growth can occur if the storage conditions of the salad are not controlled at the food producer's premises and during transport and distribution.

57.12.2.4 End-Use

The chicken and vegetable salad is a food product that is prepared for the general population. However, after purchase, abuse such as leaving the container open or keeping it at a warm temperature can cause the product to deteriorate and become a health hazard if consumed. The label should include specific conditions of storage and the use-by date.

TABLE 57.15
Hazards Associated with Processing Chicken

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Receiving chicken	Biological	N	Pathogenic bacteria (e.g. *Salmonella*, *Campylobacter*, *Yersinia*)	Supplier HACCP compliant with controls and specifications.
	Chemical	N		
	Physical	N		Cooking step
Cold storage	Biological	Y	Growth of pathogenic bacteria on long-term storage	Store below 1°C.
	Chemical	N		
	Physical	N		
Washing	Biological	N	Microorganisms may remain after washing. They redistribute when soaked	Clean sink between items.
	Chemical	N		On completion clean and disinfect.
	Physical	N	Microorganisms occur in drip water	Water control program.
			Foreign matter, unlikely	Cooking step.
				Use filtered water.
Cutting	Biological	N	Microbiological contamination from hands and equipment	Disinfect utensils and equipment.
	Chemical	N		Personnel hygiene
	Physical	N		Cooking step.
Cooking	Biological	Y	Survival of bacteria	Cook to an internal temperature of 75°C.
	Chemical	N		
	Physical	N		

TABLE 57.16
Hazards Associated with Processing Potatoes

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Receiving potatoes	Biological	N	Bacterial spores on skin.	Supplier HACCP compliant with controls and specifications.
	Chemical	N	May be grown in contaminated soil, unlikely.	
	Physical	N	Foreign matter.	Washing, cooking, and peeling steps.
				Approved and monitored supplier program.
				Wash thoroughly.
Washing	Biological	N	Microorganisms may remain after washing.	Clean sink between items.
	Chemical	N	They redistribute when soaked.	On completion clean and disinfect.
	Physical	N	Microorganisms occur in drip water.	Water control program.
			Foreign matter may remain after washing, unlikely.	Washing, cooking and peeling steps.
				Wash thoroughly.
Cooking	Biological	Y	Survival of bacteria.	Boil in water until potatoes are tender.
	Chemical	N		
	Physical	N		
Peeling	Biological	Y	Microbiological contamination from hands and equipment.	Disinfect utensils and equipment.
Trimming	Chemical	N		Personnel hygiene.
Cutting	Physical	N		

TABLE 57.17
Hazards Associated with Processing Celery

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Receiving celery	Biological	Y	Microbiological contamination	Supplier HACCP compliant with controls and specifications.
	Chemical	Y	Chemical spray residue	
	Physical	Y	Foreign matter	Wash under running water.
Refrigerated storage	Biological	N	Microbiological growth unlikely	Store below 5°C.
	Chemical	N		Inspect daily for spoilage.
	Physical	N		
Washing	Biological	Y	Microorganisms may remain after washing.	Clean sink between items.
	Chemical	Y	They redistribute when soaked	On completion clean and disinfect.
	Physical	N	Microorganisms occur in drip water	Water control program.
			Chemical residues	Use filtered water.
			Foreign matter	
Trimming	Biological	Y	Microbiological contamination from hands and equipment	Disinfect utensils and equipment.
Chopping	Chemical	N		Personnel hygiene.
	Physical	N		

TABLE 57.18
Hazards Associated with Processing Mayonnaise and Other Raw Materials

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Receiving mayonnaise and	Biological	N	Acid-resistant bacteria, unlikely	Supplier HACCP compliant with controls and specifications.
	Chemical	N	Foreign matter may remain	
ingredients	Physical	Y	undetected	Inspect before use.
Receiving trays	Biological	N	Microbiological contamination, unlikely	Supplier HACCP compliant with controls and specifications.
	Chemical	N		
	Physical	N	Foreign matter, unlikely	Inspect before use.

TABLE 57.19
Hazards Associated with the Preparation of Salad

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Mixing	Biological	Y	Contamination from hand and equipment	Clean and disinfect equipment.
	Chemical	N		Personnel hygiene.
	Physical	N		
Filling trays	Biological	N	Microbiological contamination in open	Maintain clean environment.
	Chemical	N	containers, unlikely	Close containers immediately after packing.
	Physical	N		
Storage	Biological	Y	Microbial growth on long-term storage	Store below 5°C.
	Chemical	N		Mark lot number and use by date.
	Physical	N		

57.12.3 Application of Decision Tree

Table 57.20 shows the application of a decision tree to the process steps. The four questions in the decision tree allow the identification of hazards that could be reduced at an earlier stage even though a downstream process is capable of eliminating or reducing a further risk. For example, washing potatoes removes or reduces the extent of foreign matter that may be present before they are cooked. The cooking step destroys the microorganisms. Therefore, both washing (removal of foreign matter) and cooking (eliminating microorganisms) steps are CCPs.

The decision tree is applied to all the steps to determine the CCPs. Chicken can undergo spoilage during storage. However, the storage step is not classified as a CCP because it does not eliminate or reduce the microorganisms and there is ample opportunity to inspect for spoilage during the subsequent steps of washing, cutting, and cooking. Cooking the chicken pieces for a sufficiently long period can destroy microorganisms. Hence, it is a lethal step and is classified as a CCP.

The steps of peeling and cutting after cooking can introduce contaminants from hands, knives, and other equipment. However, bacteria cannot multiply in the presence of high-acid ingredients such as mayonnaise, vinegar, pickle, etc. Therefore, the mixing step can give absolute control if sufficiently high-acid ingredients are added and thoroughly blended. Thus, the peeling and cutting steps are not CCPs.

TABLE 57.20
Critical Control Points for Chicken and Vegetable Salad

Process Step	Significant Hazard	Q1	Q2	Q3	Q4	CCP Y/N
Receive chicken	Microbial contamination	Yes	No	Yes	Yes	No
Cold storage	Microbial growth	Yes	No	Yes	Yes	No
Washing	Microbial contamination	Yes	No	Yes	Yes	No
Cutting	Microbial contamination	Yes	No	Yes	Yes	No
Cooking	Survival of microorganisms	Yes	Yes			CCP
Receive potato	Bacterial spores	Yes	No	No		No
	Foreign matter					
Washing	Foreign matter	Yes	Yes			CCP
Cooking	Survival of microorganisms	Yes	Yes			CCP
Receive celery	Microbial contamination	Yes	No	No		No
	Chemical spray					
	Foreign matter					
Washing	Microbial contamination	Yes	Yes			CCP
Trimming	Microbial contamination	Yes	No	No		No
Chopping						
Receive mayonnaise and ingredients	Foreign matter	Yes	No	No		No
Inspect ingredients	Foreign matter may go undetected	Yes	Yes			CCP
Receive trays	Foreign matter	Yes	No	No		No
Mixing	Microbial contamination	Yes	No	No		No
Storage	Microbial growth	Yes	No	Yes	No	CCP

TABLE 57.21
Control Schedule for Chicken and Vegetable Salad

Processing Step	Item to Be Controlled	Control Limit	Control Method	Frequency	Action	Responsibility	Record
Receiving Ingredients CCP2	Foreign matter	No foreign matter	Visual inspection	Every batch	Reject Inform supplier	Supervisor	Inward goods record
Cooking potatoes CCP1	Cooking temperature/ time	Cook to a minimum internal temperature of 60°C for at least 15 seconds	Monitor temperature	Continuous	Cook longer	Operator	Temperature chart
Washing Potatoes CCP2	Foreign matter	No foreign matter	Visual inspection	Every batch	Remove foreign matter Wash again	Operator	Check sheet
Trimming, chopping celery CCP2	Equipment cleanliness Foreign matter	Clean equipment No foreign matter	Visual inspection	Continuous	Remove foreign matter Clean equipment	Operator	Check sheet
Cooking chicken CCP1	Cooking temperature/ time	Cook to a minimum internal temperature of 75°C for at least 15 seconds	Monitor temperature	Continuous	Cook longer	Operator	Temperature chart
Storage filled trays CCP1	Storage temperature Labeling instructions	Store below 5°C Correct label	Monitor temperature Inspect label	Every 2 hours	Quarantine product Investigate cause	Supervisor	Temperature chart

Celery may be contaminated with foreign matter and microorganisms. The washing step will remove foreign matter but may not reduce the risk of vegetative pathogens to an acceptable level. While the reduction of foreign matter is an important process step, the trimming and chopping operations after the washing step may provide ample opportunity to ensure that foreign matter is removed. However, if there is sufficient evidence to show that the washing step does reduce the level of microbial contamination and any chemical residues to an acceptable level, the washing step could be considered as a CCP.

The plastic trays into which the salad is filled are new. Although the trays may have foreign matter, they are inspected prior to use, and hence contamination with foreign matter can be eliminated. The processes of chilling and storage of the finished product are CCPs (CCP1), and storage under controlled conditions will prevent the growth of microorganisms.

57.12.4 Overall Risk Assessment and Reduction

It is now possible to consider how risks can be reduced by making some changes to the process. Vegetables are a major source of contamination. Potato and celery can be purchased from an approved grower. Celery may be blanched or washed with chlorinated water to reduce the bacterial load. Potato can be washed, peeled, trimmed, cooked, and diced or diced and cooked. Contamination can occur during the manual handling operations, particularly after the cooking step. The use of disposable gloves during manual handling will minimize contamination. Pathogenic bacteria do not grow in acid media, and contamination during the mixing step can be controlled by adjusting the pH to 4.5 or lower. If this is not practicable, the formulation can be tested and the recipe then followed accurately. Tamper-proof or tamper-evident packs can be used as a precaution against tampering and possible contamination. The risk of abuse by the consumer can be minimized by providing warning labels that give instructions on storage after purchase. Metal detectors can be installed on line to detect metal objects in the food. The finished product can be subjected to microbiological tests to detect the presence of specific microorganisms.

57.12.5 Control Schedule

The steps that have been identified as CCPs are controlled as specified in the control schedule (Table 57.21).

57.13 CASE STUDY (II): PRODUCTION OF VACUUM-PACKED HOT SMOKED SALMON

57.13.1 Product Description

Vacuum-packed hot smoked salmon [49] is a cooked ready-to-eat product. Salmon, packed in ice, are delivered to the production facility within hours of harvesting. Salt, sugar, and

spices are bought from the market. After smoking the smoked salmon are individually packed and vacuum-sealed. They are then packed in outer boxes of polystyrene with corrugated board outside. The finished boxes are frozen at –18°C and delivered in refrigerated trucks. The shelf life of the product is one year from the production date.

57.13.2 Assessment of Hazard Potential of Vacuum-Packed Hot Smoked Salmon

The flow diagram for the production of vacuum-packed hot smoked salmon is shown in Figure 57.2. The hazards associated with the raw materials, processing steps, product, and end usage are examined next. The analysis will show the raw materials and the processes which are critical to the production of a safe product. A risk analysis is carried out to establish the CCPs.

57.13.2.1 Raw Materials

The assessment of hazard potential is shown in Tables 57.22–57.24. Fish is an ideal breeding ground for pathogens such as *C. botulinum*, *L. monocytogenes*, and parasites. Throughout the process, adequate care has to be taken to prevent the growth of microorganisms. The fish should be packed in ice until delivered to the factory. Packaging materials that come into contact with fish may harbor microorganisms. Therefore, the packaging material suppliers should have an effective food safety program in place to ensure that food-contact materials are free from biological, chemical, and physical contaminants.

57.13.2.2 Process

The hazards associated with the smoking process are shown in Table 57.23. The production facility should maintain a hygienic environment, clean and disinfected equipment, and a personnel hygiene program to minimize or eliminate the chances of contamination. The brining process has to be carried out under refrigeration because high temperatures can lead to pathogen growth. Immediately after brining, the product should be maintained below 4°C until further processing. The smoking operation is the most critical step. The smoking process should be designed to eliminate *L. monocytogenes* by holding the product at a suitably high temperature for a sufficiently long period. The internal temperature of fish should be continually monitored and recorded. If an adequate temperature is not reached, bacteria will survive. The smoking temperature of not lower than 65°C for not less than 30 minutes

TABLE 57.22
Hazards Associated with Processing Salmon and Ingredients

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Receiving salmon	Biological	Y	Pathogenic bacteria	Raw fish is frozen and the end product is also frozen
	Chemical	N	Parasites	
	Physical	N	Metal hooks	Supplier HACCP compliant with controls and specifications
				Smoking process
				Cutting step
Cold storage	Biological	N	Growth of pathogenic bacteria on long-term storage	Frozen storage
	Chemical	N		
	Physical	N		
Receiving salt and sugar	Biological	N	Foreign matter, unlikely	Inspection on receipt
	Chemical	N		
	Physical	N		
Dry storage of salt and sugar	Biological	N		
	Chemical	N		
	Physical	N		
Receiving packaging	Biological	N	Bacteria, unlikely	Supplier HACCP compliant
	Chemical	N	Foreign matter, unlikely	Inspection prior to use
	Physical	N		
Dry storage of packaging	Biological	N		
	Chemical	N		
	Physical	N		
Weighing salt and sugar	Biological	Y	Microbiological contamination from hand and equipment	Personnel hygiene
	Chemical	N		Clean and disinfect equipment
	Physical	N	Chemical residues	Cleaning procedures
Mixing	Biological	N	Microbiological contamination from hand and equipment	Personnel hygiene
	Chemical	N		
	Physical	N		

TABLE 57.23
Hazards Associated with Processing Steps

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Semi-defrosting	Biological	Y	Pathogenic bacteria growth, unlikely since the fish is only semi-defrosted	Monitor semi-defrosting
	Chemical	N		
	Physical	N		
Cutting and trimming	Biological	Y	Contamination from hands	Personnel hygiene
	Chemical	N	Foreign matter	Inspection
	Physical	N		
Brining	Biological	Y	*C. botulinum* growth and toxin production in finished product	Proper brining
	Chemical	N		Correct salt concentration
	Physical	N	Other bacterial pathogens	
Rinsing	Biological	N	Microbial contamination, unlikely due to short time	Rinsing procedure
	Chemical	N		
	Physical	N		
Drying	Biological	Y	Microbiological concentration; salt content in the fish is insufficient to inhibit growth	Controlled at the smoking step
	Chemical	N		
	Physical	N		
Smoking/ cooking	Biological	Y	Survival of pathogens	Proper smoking/cooking
	Chemical	N	Due to inadequate cooking	
	Physical	N		
Cooling	Biological	N	Growth of pathogens	Cooling procedure
	Chemical	N		Combination of salt and smoking
	Physical	N		Too short a period for growth to occur

TABLE 57.24
Hazards Associated with Packing Hot Smoked Salmon

Processing Step	Hazard Type	Significant Y/N?	Hazard Description	Control Method
Vacuum packing/ labeling	Biological	Y	Introduction of bacteria	Packing procedure
	Chemical	N	Temperature abuse can allow growth during subsequent distribution and storage	Indicate storage conditions (–18°C) on the label
	Physical	N		
Boxing	Biological	N	Microbiological contamination, unlikely	Packing procedure
	Chemical	N		
	Physical	N		
Cold storage	Biological	Y	Growth of *C. botulinum*	Correct storage temperature
	Chemical	N		
	Physical	N		
Distribution	Biological	N	Bacterial growth, unlikely	Polystyrene boxes and refrigerated trucks
	Chemical	N		
	Physical	N		

is based on the coldest part of the fish in the oven. Severe temperature abuse during packaging, storage, and distribution can allow pathogen growth. The storage conditions should be specified on the label so that consumers are aware of the danger of storage outside the stated limits.

57.13.2.3 Product

The drivers must ensure that refrigerating units in delivery trucks are maintained in good working condition. Microbial growth can occur if the storage conditions are not adhered to at the food production facility and during transport and distribution.

57.13.2.4 End Use

The product is intended for general use. The label must comply with regulatory requirements and should specify any colorants or additives added, storage conditions, and the expiry date.

57.13.3 Risk Assessment

Raw fish may carry pathogenic bacteria and parasites. Risk assessment of the hot smoking process depends on the severity of the outcome and the likelihood of occurrence of the hazard in the food product. Throughout the process, the

TABLE 57.25
Control Schedule for Hot Smoked Salmon

Activity	Potential Hazard	Critical Limit	S	L	A	Control Method	Corrective Action	Person Responsible	Record
Receiving salmon	Microbial growth	Received frozen	2	D	12	Product is frozen Supplier HACCP compliant Controlled at smoking	HOLD, inform supplier	Supervisor	Inward goods receipt
Weighing salt and sugar	Microbial contamination	No contamination	3	D	17	Personnel hygiene Disinfect equipment	HOLD product, monitor cleaning and hygiene	Supervisor	Check sheet
Semi-defrosting	Microbial growth	No growth	2	D	12	Defrosting procedure Controlled at smoking	HOLD, investigate	Supervisor	Temperature chart
Cutting and trimming	Microbial contamination	No contamination	2	D		Personnel hygiene Controlled at smoking	HOLD Check cleaning and hygiene	Supervisor	Check sheet
Brining **CCP**	Microbial growth	Minimum 24-hour soaking Minimum salt Content of 6%	2	C	8	Time in brine Salt content Weight of fish and brine Maintain chill room temperature	HOLD longer in brine Investigate	Supervisor	Check sheet Temperature chart
Drying	Microbial concentration	No growth	3	D	17	Correct drying procedure Correct salt concentration	HOLD Investigate	Supervisor	Check sheet
Smoking **CCP**	Survival of microorganisms	No microorganisms	2	C	8	Minimum internal temperature of fish of 65°C for 30 minutes	HOLD product Investigate	Smoke Operator	Temperature chart
Vacuum packing labeling	Growth of microorganisms	No growth	3	C	13	Presence of appropriate label statement on storage Correct packing procedure	Relabel	Packing Supervisor	Check sheet
Cold storage **CCP**	Bacterial growth Toxin production	No growth or toxin	2	C	8	Cooler temperature of 0°C or less	HOLD product Adjust cooler temperature	Supervisor	Temperature chart

Note: S = Severity; L = Likelihood; A = Assessment.

growth of pathogenic bacteria such as *C. botulinum* and *L. monocytogenes* is a potential hazard that can lead to serious illness (severity score = 2). The likelihood of provoking the illness is extremely low [50]. The pathogenic organisms grow at temperatures above 1°C, and they compete with spoilage flora which grows well at temperatures below 1°C. Therefore the fish is likely to be spoiled before the production of toxin or the development of a high number of pathogens. When the products are cooked before consumption, the risk is completely eliminated. Thus, the likelihood at the fish receiving step is classified as D (unlikely to occur).

Brining is an essential part of the whole operation as the salt content in the fish in combination with smoke and heat treatment is necessary to control the growth of microorganisms. There have been reported cases of contamination of smoked salmon with *L. monocytogenes* [51]. Because of the critical nature of brining, smoking, and cool storage operations and reported cases of contamination, the hazard of microbial growth is classified as a likely event (likelihood score = C) and thus, these steps are CCPs. Table 57.25 shows the risk analysis of the hazards identified as significant.

57.13.4 Overall Risk Assessment and Reduction

The fish are a major source of microbial contamination. Raw fish should be examined for freshness and wholesomeness. All eviscerated fish or fish not being used should be kept below 0°C. Frozen fish should be checked for wholesomeness. The production facility should ensure that the cleanliness of plant and equipment, a hygienic environment, and personnel hygiene are maintained to minimize the risks of contamination.

During frozen fish storage, the temperature can be monitored through continuous surveillance using security alarms. The smoking process is critical, and the internal temperature of fish during this process can be monitored using thermocouple probes in the three thickest fish in the coldest part of the oven. A sample from each oven load should be analyzed for water-phase salt (WPS) level. The cooling step after smoking should be controlled because of the possibility of microbial growth if the cooling period is too long.

Time–temperature integrated (TTI) labels may be applied to the finished product to indicate any abuse of temperature over a certain period of time. Tamper-evident packaging will provide further security against intended or unintended contamination. An automatic labeling system with alarms will ensure the application of the label on every pack.

57.13.5 Control Schedule

The steps that have been identified as CCPs are controlled as specified in the control schedule (Table 57.25).

57.14 GOOD MANUFACTURING PRACTICES

In preparing the salad and the hot smoked salmon there are several operations that are control points but not critical control points. These steps can be controlled by implementing good manufacturing practices. Inspection and storage of raw materials, cleaning of equipment, use of food-grade detergents and disposal gloves, training in personnel hygiene, a preventive maintenance program, and the use of status stickers are some of the controls that must be considered to assure the safety of the final product. Regular audits provide a means of verifying the effectiveness of the procedures.

57.15 ISO 22000 STANDARD

The International Organization for Standardization developed the ISO 22000 Food Safety Management Standard [52]. It will define the requirements for a food safety management system for all organizations in the food chain from farmer to catering, including packaging. The standard aims to harmonize the voluntary international standards.

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58 Good Manufacturing Practice (GMP)

Titus De Silva

CONTENTS

58.1 INTRODUCTION

Good Manufacturing Practices (GMPs), also known as current Good Manufacturing Practices (cGMPs), are a series of manufacturing and administrative procedures aimed at ensuring that products are consistently made to meet specifications and customer expectations. The regulations governing GMP cover a variety of consumer goods such as human pharmaceutical products and veterinary products (21 CFR 210-211); biologically derived products (21 CFR 600 and CFR 620); medical devices (21 CFR 820); manufacturing, packaging, or holding human food (21 CFR 110); and processed food (21 CFR 100) [1]. In relation to food, the implementation of GMP results in safe and quality food. In the United States, the Food and Drug Administration (FDA) has issued these regulations as the minimum requirements for manufacture. Most countries have their own GMP regulations for pharmaceuticals. The first formal set of GMPs was published by the FDA in 1963 as Part 133 [1].

58.2 GMP ACTIVITIES

Figure 58.1 shows the various activities of GMP. It supports and brings together many programs, systems, and philosophies that lead to an effective food business providing safe and quality products. The three main elements of GMP are

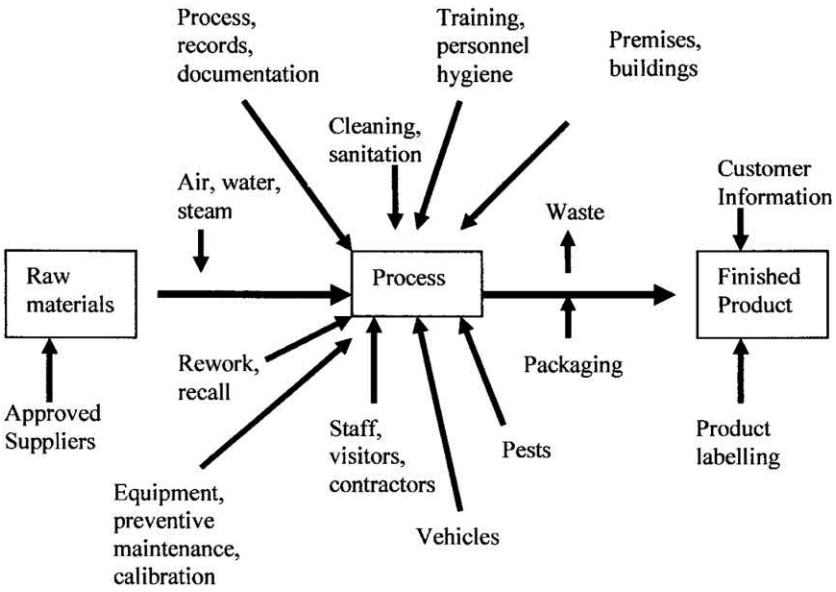

FIGURE 58.1 Activities of GMP.

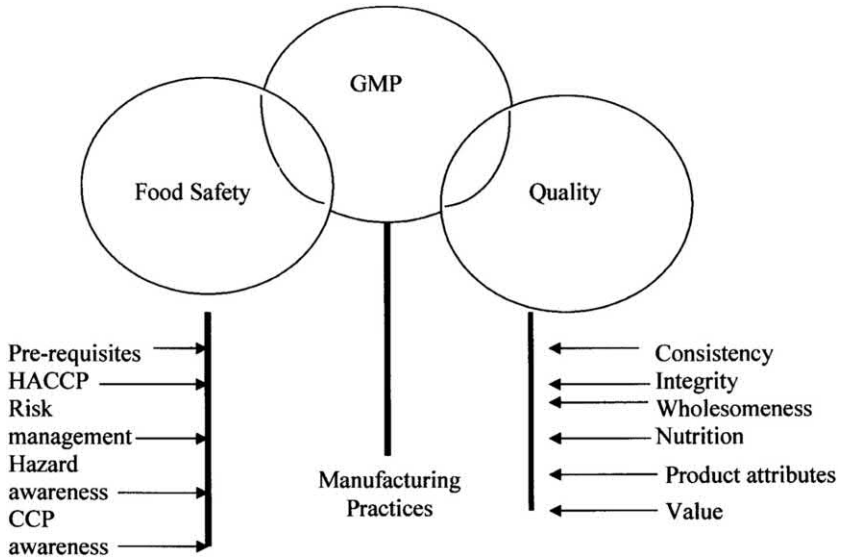

FIGURE 58.2 Core food business.

food safety, good practice, and quality. Their relationship to one other is shown in Figure 58.2. Food safety and quality management systems, and the standards provide a firm foundation for the survival of the business by adding attributes of quality and value to the product. This will enable the food business to gain competitive advantage.

58.3 GMP PHILOSOPHY

The philosophy behind GMP is summarized [2] as (i) GMP is closely aligned with disciplines such as quality, management, food safety, and food quality; (ii) GMP is designed by food manufacturers for food manufacturers; (iii) GMP involves the entire food business operation from establishment of policy to its implementation; (iv) GMP is a proactive and hands-on document; (v) GMP has provision to exceed customer's expectations and provides confidence in the product and consistency in the process; (vi) every activity within the food business impacts on the finished product; and (vii) through GMP, value is built into the product and loyalty to the brand.

58.4 FOUNDATION OF GMP

GMP is based on two complementary and interacting components: effective manufacturing operations and effective food control operations. Complementary to these two components are the management functions of these two components.

58.4.1 Effective Manufacturing Operations

GMP requires that every aspect of the food manufacturing process is clearly defined and effective in achieving the desired result and that all necessary facilities are provided, including [3] (i) adequate premises and space; (ii) correct and properly maintained equipment; (iii) appropriately trained personnel; (iv) correct ingredients and packaging material; (v) suitable storage and transport; (vi) documented procedures for all operations including cleaning; (vii) appropriate management and supervision; and (viii) adequate administrative, technical, and maintenance support. Record keeping is an integral part of the manufacturing operation, and records provide evidence of completed activities in the plant. Procedures are necessary for all processes including training, written in a manner easily understood by the operators.

58.4.2 Effective Food Control

Effective food control can be achieved by having an efficient food control management plan by (i) providing adequate facilities for inspection, sampling, and testing; (ii) monitoring process conditions and the production environment; and (iii) providing prompt feedback to manufacturing personnel to enable them to make adjustments, when necessary. Control mechanisms are established for all tasks from receiving raw materials to the delivery of finished goods or disposal of nonconforming goods.

58.4.3 Effective Management

The achievement of GMP objectives is the responsibility of senior management and requires active participation and commitment of personnel in many interacting departments at all levels within the organization and its suppliers. To accomplish the objectives, there must be a comprehensively designed and effective quality system incorporating the elements of GMP. The basic concepts of quality assurance, GMP, and quality control are interrelated. Senior management must have a formal commitment to compliance with the GMP requirements. Management has to ensure that the key personnel are suitably qualified and trained to carry out their tasks according to the prescribed procedures. Over the past 10 years, there has been a global attempt to formalize the management process that guides the establishment of GMP [4]. The result of this attempt is the ISO 9000 series of standards.

58.5 PRELIMINARY PROCESS

A manufacturing organization adopting the GMP principles is able to (i) consistently meet its own and customers' requirements, (ii) meet industry standards and codes of practice, and (iii) comply with regulatory requirements. In order to achieve this, the organization has to plan its activities. The preliminary process for GMP is shown in Figure 58.3. The scope of GMP is not only the production of a perfect end product but also the demonstration of activities that accomplish the end product.

58.6 GMP ACTIVITIES

GMP is intended to build quality into the product at all stages of the operation, and the activities associated with GMP are [5, 6] (i) organization and personnel, (ii) training and personnel hygiene, (iii) building and facilities, (iv) equipment, (v) control of components, (vi) production and process control, (vii) packaging and label control, (viii) storage and distribution, (ix) laboratory control, (x) documentation, (xi) cleaning and sanitization, (xii) maintenance and calibration, (xiii) pest management, (xiv) foreign matter control, (xv) waste management, (xvi) reworking material, (xvii) audits and review, (xx) customer complaint procedure, and (xxi) recall procedure.

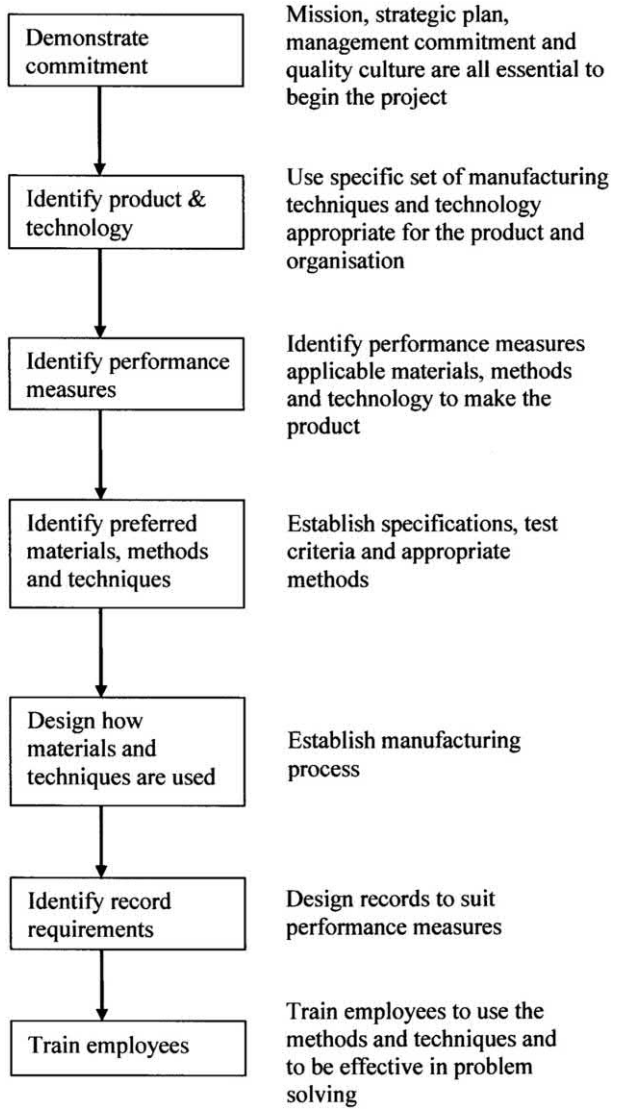

FIGURE 58.3 Preliminary process for GMP.

58.6.1 Organization and Personnel

To implement the GMP program effectively, it is necessary for the organization to establish a structure appropriate for the business. It must have an adequate number of personnel who have the relevant qualifications and training to perform the required tasks. Their duties should be clearly explained before the commencement of the tasks. The training program should cover the principles of GMP, specific tasks, and personnel hygiene.

The three key personnel in a manufacturing organization are the quality control/quality assurance (QC/QA) manager, the production manager, and the purchasing manager. The QC/QA manager has the authority to (i) approve materials and finished goods; (ii) reject, hold, or quarantine nonconforming products; and (iii) recommend suppliers. The production manager is responsible for production, personnel, equipment, operations, and the management of records. It is the responsibility of the production manager to ensure that products are manufactured to the relevant specifications within the budgeted cost. In consultation with the QC/QA manager, the production manager should (i) generate specifications for materials and products, (ii) train manufacturing staff, and (iii) control the manufacturing environment and hygiene.

The purchasing manager has the authority to order raw materials that comply with established specifications and initiate action with the suppliers when the purchased product does not meet product specifications. The management must demonstrate support and commitment to the principles of GMP and create an environment where the employees take ownership. Leadership and motivation are key features of an effective business organization. The managers must adopt a proactive approach and promote a quality culture within the organization so that the employees take pride in their workmanship.

58.6.2 Training and Personnel Hygiene

The organization has to ensure that the employees have appropriate facilities and are trained to promote personnel hygiene and safe food handling. The training program has to reflect the type of operation and the level of education of its employees.

58.6.2.1 Recruitment and Induction

The basis of the recruitment and selection process is the ability to do the task at the time, unless the organization has a program to train the new recruit. The organization has to ensure that the person has the capability to perform the job either after training or with previous experience. The recruitment process and subsequent training are thus critical for food safety. Job descriptions have to be established for all positions. The relevant competence and/or training requirements

are specified for each job. The aim of the induction process is to prepare the new employee for the company culture and the process itself should commence with the introduction of the person to the business, its quality system, organization expectations, and the tasks required of them. The person's responsibilities, reporting structure, and the disciplinary process have to be clearly defined.

58.6.2.2 Competence and Training

Competence is the demonstrated ability to apply knowledge and skills to a task correctly and completely the first time without assistance. The organization should ensure that minimum levels of competence are maintained and that the employees perform the tasks without supervision.

The training program should include the principles of GMP and the demonstration of competence in the company's hygiene practices, its quality management system, and the operating principles of the activity. It is essential that the trainer emphasizes the reason for maintaining food hygiene and compliance with legal requirements. At regular intervals, it is the manager's responsibility to review the training requirements and address the deficiencies.

58.6.2.3 Food Hygiene Requirements

Training in food hygiene is an essential part of the training program. The food hygiene requirements [7, 8] include (i) clean protective clothing; (ii) hair and beard covers where necessary; (iii) washing hands before commencing work; (iv) keeping the body, hands, and clothing clean; (v) prohibition of smoking within the premises; (vi) exclusion of personnel suffering from infectious disease; (vii) covering wounds with waterproof plaster; (viii) first aid material; and (ix) prohibition of wearing jewelry or loose items.

58.6.3 Building and Facilities

Buildings should be located, designed, and constructed to suit the type of operation that permits the separation of individual processing, manufacturing, packing, testing, and storage operations to avoid mix-ups, cross-contamination, and deterioration. At all times the premises should be maintained in a clean and tidy condition. The processing areas should have (i) a ceiling made out of materials that prevent mold growth or build up of dust; (ii) floors made of impervious material free from cracks or open joints; (iii) walls of smooth impervious material to facilitate easy cleaning; (iv) environmental controls such as adequate lighting, ventilation, heating, cooling, and proper washing and sanitary facilities; (v) entry points sealed off to prevent pest access and inhibit waste odors; (vi) drains that allow maximum flow of waste and have trapped gullies and proper ventilation; (vii) changing rooms segregated from processing areas; (viii) provision to contain waste in covered containers regularly disposed; (ix) protection for receiving and dispatch of materials and products in storage from weather; (x) written procedures for cleaning all processing areas; and (xi) pest control devices placed at appropriate locations away from open product.

58.6.4 Equipment

All equipment used in the manufacturing area should be suitable for its intended use and properly maintained and cleaned. It is a good practice to use protective covers on equipment where there is a threat of contamination from external sources. All precautions must be taken to prevent contamination from hands and equipment during maintenance when processing takes place. All surfaces which come into contact with food should be (i) inert to the food and should not contaminate the food; (ii) microbiologically cleanable, smooth, and nonporous; (iii) visible for inspection and cleaning; and (iv) set to enable self-emptying or self-draining. The equipment should be arranged to protect the contents from external contamination from leaking joints, lubricants, and broken metal parts. A program of preventive maintenance should be scheduled for all equipment, and during preventive maintenance it is essential that the engineer reports the missing parts such as nuts, bolts, springs, etc. so that appropriate action can be taken. Before and after every use, it is a good practice to clean all equipment. Documented cleaning procedures should be available at all workstations. Power-driven equipment can sometimes generate fumes and appropriate precautions should be taken to prevent the food from contamination.

58.6.5 Control of Components

To ensure the finished product has the right quality attributes and is safe to consume, the raw materials, including packaging that comes into direct contact with food are purchased from approved suppliers. The criteria for the approval of suppliers should include the following: (i) the supplier should be able to meet the organization's requirements, (ii) the quality is acceptable, (iii) the supplier can meet the delivery needs, (iv) the price is acceptable, and (v) the supplier has an effective food safety program in place.

Adequate precautions should be in place to prevent any contamination before and after delivery to the premises. They are held in a segregated area in quarantine until their status has been determined after appropriate inspection. Segregation should also be extended to electronic documentation to prevent the material from being used in production before their status has been determined. The organization's inspection criteria may include (i) conformance certificates, (ii) provision of test results, (iii) identification on receipt, and (iv) full analysis or any combination of these criteria. While in storage, all raw materials are regularly checked to ensure that they remain in acceptable condition. The storage areas are maintained in hygienic condition and in conditions appropriate for the type of raw material.

During the receipt and issue of raw materials, the organization has to ensure that the details of products are traceable through appropriate record keeping. Further controls may include checking weights/volumes, stock rotation and identification. It is essential that appropriate stickers are applied following inspections. The status stickers generally in use are

HOLD: materials whose status is in doubt
QUARANTINE: materials whose status is yet to be determined
REJECT: materials unsuitable for use
PASSED: materials suitable for use

The reasons for the hold, quarantine, or rejection and the date should be clearly stated on the label.

58.6.6 Production and Process Control

A fundamental requirement of any manufacturing organization is that the processes are capable of consistently producing finished goods, which conform to established specifications, and for food products to be free from contaminants. The effectiveness of the processes have to be determined prior to production by means of controlled trials. Similar evaluations are done whenever there is a change in the raw materials, methods of manufacture, or equipment. Before commencing production, checks should be carried out to ensure that (i) the processing area is free from potential contaminants, (ii) correct materials and methods are used, (iii) correct setup procedures are used, and (iv) all equipment is clean and ready for use. Work instructions are kept at all workstations and are written in a clear and simple style for easy comprehension by the operators.

58.6.6.1 Controls during Production

Accurate records have to be maintained of all production data including the operating conditions and quality parameters. Statistical control charts are used to identify deviations from the normal. Problems that cause stoppages, breakdowns, and emergencies are identified, recorded, and promptly addressed. Process controls specify the type of tests to be conducted, the frequency of checks, and the operating limits. Suitable methods should be used to identify the batches of production, delivery vehicles, container numbers, etc., to enable effective traceability. The controls ensure that the product is not contaminated and is capable of preventing the growth of microorganisms. This is achieved by careful monitoring of physical factors such as time, temperature, humidity, water activity, pH, pressure, flow rate, and others as appropriate. Suitable conditions are maintained throughout the process to prevent the decomposition of the product and/or the growth of microorganisms.

During processing, steps are taken to ensure that food is protected against inclusions of metal and other extraneous material. This can be achieved through the use of magnets, metal detectors, etc. Food processing operations often involve processes such as washing, peeling, cutting, sorting, inspecting, etc., and these are performed in a manner that prevents the food from contamination from hands, equipment, utensils, etc. The processing areas used for food production are not used for the manufacture of nonhuman food.

58.6.6.2 Control of Finished Product

Finished products are not released until they are tested and approved by the authorized person. Nonconforming products are segregated and quarantined. Appropriate storage conditions are adhered to before and during the delivery of finished goods.

58.6.7 Packaging and Delivery

Very often in packaged food items, the packaging material comes into direct contact with the food. The organization must ensure that packaging materials possess the following features: (i) made with approved food-grade material; (ii) capable of protecting the food item during its expected life under normal conditions; (iii) appropriate for the production in terms of moisture absorptions, moisture loss, etc.; (iv) capable of standing up to recommended operations such as microwave heating, pressure, etc.; (v) no interaction between the food and the packaging material; (vi) capable of maintaining the characteristics and the integrity of the product; (vii) correct for the product being manufactured; and (vii) passes the test criteria.

Over the last few years, consumers have become increasingly aware of the characteristics of the products they purchase through effective communication. The regulatory measures ensure that appropriate information is displayed on food products either on the label or on the immediate package. The label is not only a marketing tool but also a source of information to the consumer. The finished product should carry mandatory information and other information such as instructions for reconstitutions, storage conditions, target consumer groups, etc. as applicable. A unique code identifies the food product for recall purposes, if necessary. In many countries, attention is now focused on the declaration of allergens, and whether the food is genetically modified.

58.6.8 Storage and Distribution

Storage and distribution are significant elements in the food supply chain. The primary purpose of storage and distribution of food products, ingredients, and packaging is to protect them against physical, chemical, and microbial contamination as well as against deterioration of the food and the container. The principles of good hygiene practice apply not only to food and the ingredient but also to packaging components that come into direct contact with food. The buildings, grounds, and equipment of food storage warehouses have to be designed, constructed, and maintained in a manner that does not compromise food safety standards.

58.6.8.1 Storage

Storage, transport, and distribution are an important link between the producer and the consumers. Even if sufficient care has been taken to ensure the quality and the safety of food, uncontrolled storage and transport conditions can severely affect food products. The storage conditions have to be appropriate for the type of food production facility, and the controlled conditions include (i) regular inspection of material for signs of deterioration, (ii) maintaining correct storage conditions for the type of product, (iii) regular cleaning

to maintain storage areas in hygienic conditions, (iv) a pest control program, (v) maintaining stock rotation, (vi) maintaining the integrity of the stock during internal and external transport, and (vii) segregation of products that have not been released for distribution.

58.6.8.2 Distribution

It is essential that transport and distribution are carried out by approved suppliers. The organization should ensure that vehicles used for transporting food products are not used for carrying animals, harmful articles, chemicals, or biological products. It is a good practice to ensure that the transporting facility has a good cleaning program, and it is the responsibility of the organization to inspect vehicles for general cleanliness, accumulation of water, presence of foreign material, or damage that could cause contamination of the food product. The inspection checklist may include (i) openings that permit the entry of pests, rodents, birds, and/or insects; (ii) foreign odors; and (iii) presence of nails splinters, oil, grease, dirt, and/or bird droppings. Security precautions should deter any tampering with products during transport. Distributing the load in a uniform manner can avoid damage to the products and people or the vehicle during transport. All instruments necessary to maintain the environmental conditions inside the vehicle should be regularly inspected and maintained.

58.6.9 Laboratory Controls

Laboratory testing is an integral part of the production process and it provides its customers, both internal and external, with accurate test results at all relevant times in order to fulfill the GMP requirements. The minimum requirements for laboratory practice are shown in Table 58.1. It is important to maintain the integrity of the testing laboratory. This may be achieved by the accreditation of the laboratory to a national standard.

TABLE 58.1
Requirements for Good Laboratory Practice

Control Item	Requirement
Personnel	Qualified and competent
	Suitably trained
Sample control	Documented criteria for receiving, accepting, and rejecting samples
	Clear traceability information
	Procedure for handling and storage of samples
	Sampling plans and equipment for sampling
Facilities and environment	Facilities and environment compliant with regulations
	Good housekeeping
	Monitored environmental conditions
	Prevention of cross-contamination
	Appropriate facilities and environment for conducting tests
Equipment control	Suitable equipment for effective performance of tests
	Calibration schedule for equipment
	Maintenance of current equipment
	Procedure for the purchase of new equipment
	Clear operating instructions at workstations
	Reference materials such as weights, thermometers, hydrometers, etc.
Test methods	Validated and documented test methods
	Performance monitoring
	Validated quality control standards such as media, pH, buffers, etc.
	Procedure for "suspect" test results
Record control	Management of records of test results
	Traceability of a record to a standard/sample
	Inclusion of information such as the operator, signature of the authorized person for approval, and amendments and deviations from expected results
	Preserved from deterioration
	Easy retrieval
Test reports	Testing laboratory name and the customer
	Sample details
	Test method
	Accuracy of the test method
	Appropriate dates
	Authorized signature
	Results of tests as required by the customer
Documentation control	Scope of the laboratory function
	Recruitment of staff and necessary qualifications
	Management of samples
	Management of equipment
	Test methods
	Performance reviews
	Reviews of corrective action reports and audits

58.6.10 Documentation

The documents in the production facility provide evidence that a particular activity has been followed and has been successful or failed. The aims of documentation are as follows: (i) define materials, processes, control measures, and products; (ii) record and communicate information needed before, during, and after production; (iii) reduce the risk of error arising from poor communication; and (iv) permit investigations and traceability in case of failures of product or process. Documents fall into three main categories: instruction and procedures, programs, and records and reports.

58.6.10.1 Instructions and Procedures

Instructions and procedures are essential for any plant's effectiveness and efficiency. They provide information about how to perform tasks safely, effectively, and efficiently. They can be used for the training of employees. Instructions and procedures incorporate the best practices that result from previous learning experiences. The user is actively involved in the development of procedures and instructions and they have to be helpful and intelligible. A good procedure (i) describes the purpose of the task, (ii) emphasizes significant steps sequentially, (iii) defines responsibilities, (iv) provides guidance in the case of a problem and identifies decision points, and (v) is supported by flow diagrams, photos, and drawings in color. The documents that provide instructions and procedures are shown in Table 58.2.

TABLE 58.2
Instructions and Procedures

Document	Description
Specifications	Raw materials, packaging materials, in-process controls, finished goods, customer requirements
Procedures	Manufacturing instructions
	Purchase procedures
	Quality policy and quality system procedures
	Product recall procedures
	Standard operating procedures
	Cleaning instructions
	Plant operating instructions
Schedules	Maintenance schedule
	Calibration schedule
	Audit schedule
	Review schedule

58.6.10.2 Programs

Programs are the scheduled activities carried by the organization to meet its targets. These include production programs, training programs, pest management programs, waste disposal programs, and environmental management programs.

58.6.10.3 Records and Reports

The records and reports include data entered before, during, and after manufacture to provide evidence of controls in place for the production of safe food. Some examples of records and reports are (i) records of receipt, inspection, approval, and issue of raw materials; (ii) records of tests and release information on bulk products, finished products, and intermediates; (iii) in-process control charts and calibration records; (iv) weight/volume control records; (v) batch manufacturing records; (vi) customer complaint reports; (vii) quality control records, audit reports, and reviews; and (viii) corrective action reports. Documents and records are maintained for a period after the end of the expected shelf life of the product as specified by the regulatory bodies or as required by the organization. These also include electronic data and documents that should be backed up according to a scheduled program.

58.6.10.4 Document Control System

An effective documentation control system ensures that the documents and critical control records are available, up to date and correct. The following information is essential for a document control system: (i) title, (ii) issue date and issue number, (iii) signature of the authorized person, (iv) reference number of the document, (v) page number, and (vi) changes made.

58.6.11 Cleaning and Sanitation

Cleaning is the complete removal of food soil using appropriate chemicals as recommended. Sanitation is the process of reduction of microorganisms to a safe level. Cleaning and sanitation is the most important aspect of the hygiene program. Detailed procedures have to be established for all surfaces that come into contact with food products as well as for others such as overhead structures, walls, ceilings, etc. The objective of any cleaning and sanitization program is to remove food soil on which microorganisms can grow and kill microorganisms present. The cleaning program has to be evaluated for its effectiveness by carrying out appropriate tests. The procedures should specify the cleaning and type of chemical for each production line.

58.6.11.1 Cleaning and Sanitization Steps

Cleaning and sanitization consist of four steps [9, 10]: (i) Physically remove food particles and foreign matter by sweeping, wiping, or prerinsing in cold water. Rinse any equipment containing high-protein substances with cold water (hot water will cause proteins to be baked onto surfaces). (ii) Wash equipment in hot water and detergent to remove grease and dirt. (iii) Rinse with hot water again to remove dirt and detergent, and air-dry. (iv) Sanitize equipment and surfaces with a chemical or by immersing in water at 82°C.

58.6.11.2 Cleaning Methods

Mechanical cleaning, also referred to as cleaning-in-place (CIP), employs automated programs. It does not require disassembly or partial assembly. The temperature and contact times are automatically controlled. Cleaning-out-of place (COP) allows partial disassembly and involves cleaning in specialized pressurized tanks. Manual cleaning requires total disassembly for cleaning and inspection.

58.6.11.3 Sanitization Methods

Effective sanitization of food product contact surfaces achieves a reduction of contamination level by 99.999% (5 logs) in 30 seconds [10]. Nonproduct contact surfaces require a contamination reduction of 99.9% (3 logs) using the test organisms *Staphylococcus aureus* and *Escherichia coli*. There are two types of sanitization methods: (i) thermal sanitization, which involves the use of hot water or steam at a specific temperature for a specific contact period; and (ii) chemical sanitization, which involves the use of an approved chemical sanitizer at a specified concentration and contact time.

58.6.12 Maintenance and Calibration

Repair, maintenance, and calibration of the plant and equipment are essential for the effective operation of the processes.

58.6.12.1 Repair

When repairs are done by outside contractors, such as electricians, fitters, builders, plumbers, etc., the standard of service required has to be specified. The organization must ensure that they comply with food hygiene and other requirements, and that the work has been carried out to the expected standard.

58.6.12.2 Preventive Maintenance

An effective preventive maintenance program will avoid unnecessary downtime due to machine breakdowns. Suitably

qualified and skilled personnel have to be employed to carry out maintenance activities. In addition to the preventive maintenance schedule, the management must respond to breakdowns or unusual occurrences observed or detected by the operators.

58.6.12.3 Intrusive Maintenance

Intrusive maintenance is the activity of carrying our maintenance while production is in progress. When intrusive maintenance is carried out, the safety of the product is not compromised. Adequate precautions have to be taken to ensure that the product in progress is carefully controlled and protected to prevent any contamination as a result of maintenance or change in environmental conditions.

58.6.12.4 Calibration

It is necessary to calibrate measuring equipment to maintain the integrity of the measurements. Calibrations are carried out by certified organizations to enable traceability to a national standard. The calibration schedule has to reflect the critical nature of the measurement, the environment in which the instrument is placed, and the previous history of deviations. The equipment and instruments that are out of calibration should be promptly removed from use.

58.6.13 Pest Management Program

The most effective method of controlling infestation is by maintaining good housekeeping standards. Control of pests is important to prevent the spread of disease and contamination of the product. To maintain the pest management program, it is essential to have appropriately trained personnel or employ a professional pest control organization. A documented pest management program contains the following information: (i) location of baits; (ii) the date when the baits are placed; (iii) record of use (quantity, areas, frequency) of pesticides; (iv) chemicals used for rodents, birds, and insects; (v) record of signs of pest activity; and (iv) procedure for updating the program when the system or building changes.

The program manager ensures that all materials, products, packaging, utensils, and surfaces in contact with food are protected from contamination by pest control substances. An effective system of internal communication is essential so that the production personnel can prepare the plant for pest control. Products and equipment are stored at a distance of 50 cm from adjacent walls to facilitate cleaning and inspection for infestation. Site inspections are carried out at regular intervals and records of inspections are maintained as evidence.

Pest control devices include electric fly killers, rodent traps, nets for birds, scaring devices, shooting of birds, baits, and insecticides. The entry of pests can be prevented by (i) careful design and maintenance of buildings to prevent any access, (ii) clearing of nesting sites, and (iii) providing appropriate screens on doors and windows. A good housekeeping program reduces the risk of infestation. This is achieved by (i) effective waste management, (ii) inspection of food products on receipt, (iii) proper storage in pest-proof environment, and (iv) closing all possible entry points.

58.6.14 Foreign Matter Control

Foreign matter can originate from incoming goods and during processing operations. In order to minimize the risk of contamination, controls are applied at various locations in the production chain, i.e., supply, manufacturing, packaging, storage, and distribution.

58.6.14.1 External Sources of Contamination

The external sources are often associated with contaminants such as pests. Similarly, methods of handling, production, and packaging can give rise to foreign matter, for example, stones in peanuts and pieces of wood in herbs. Since raw materials are the primary source of contamination, the specifications should be designed to include limits on the exclusion of foreign matter.

58.6.14.2 Internal Sources

There are several internal sources of foreign matter: (i) building, installation, plant, and equipment; (ii) surface coatings and finishes; (iii) maintenance tools; (iv) personnel; and (v) recovered product.

58.6.14.3 Foreign Matter Control

Plant, equipment, and buildings are regularly inspected to detect and address any deterioration, soiling, or detachments. During processing, precautions can be taken to minimize the risk of contamination with foreign matter and such precautions include (i) the use of metal detectors; (ii) sieving, filtering, sifting, washing, inspection, and sorting; (iii) magnetic grids and plates; (iv) keeping containers not in use inverted; and (v) closely monitoring glass breakages and implementing an effective clean-up procedure.

Implementing a Hazard Analysis and Critical Control Points (HACCP) program minimizes the potential for contamination of food with foreign matter, chemicals, and microorganisms.

58.6.15 Waste Management

With the global focus on environmental management, waste and effluent disposal have become important issues for organizations as well as for regulatory bodies. The organization has to ensure that waste is controlled to ensure that there is no contamination to the finished product. Contamination could occur from spills, waste plastic drums/containers, harmful chemicals, etc. Food scraps are also a major source of food for bacteria, insects, and rodents. Packaging may be a source of food. A well-planned waste management program with procedures, accountabilities for those managing waste, and accurate records will minimize not only regulatory noncompliances but also chances of contamination. ISO 14001 [11], the Environmental Management System, is an ideal standard to manage the environment. Table 58.3 provides guidelines for managing waste [12].

TABLE 58.3
Guidelines for Managing Waste

Item	Guideline for Managing
Waste bins	Require covers and locate well away from food production and storage areas
	Easily accessible for removal
Wet refuse	Metal or plastic containers with tight-fitting lids
	Collected from the premises daily or if this is not possible, precautions are taken to prevent rotting and pest access
	Regularly cleaned in suitable washing areas
Dry refuse	Metal or plastic containers with tight-fitting lids or in paper or plastic sacks held firmly with a close-fitting lid
Yard areas	Kept clean and dry
	Areas designated for storage of waste are included in the cleaning schedule
Waste on the floor	Cleaned immediately
Raw material packaging	Not used for storing food products
	Empty packaging disposed immediately
Old food containers	Not used for storing chemicals or nonfood items
	Label contents of the container
Emergencies	Establish emergency response procedures
	Contact numbers readily available
Environmental spills	Environmental spill kits

58.6.16 Reworking Material

Defective products may be reworked or reprocessed provided the resultant product complies with relevant specifications and is safe for consumption. Procedures should clearly define the standards for reworking or reprocessing. If defective products cannot be reworked, rejection may be the only viable option. All rejected products must be clearly labeled and physically segregated. The manner of disposal depends on the nature of the rejected product, but the organization has to consider (i) the means of recovering the cost, (ii) safeguarding the reputation of the company, (iii) protecting the consumer, and (iv) compliance with regulatory requirements.

58.6.17 Customer Complaint Procedure

Governments have enforced regulatory controls to ensure the rights of consumers. Customer complaints can be a valuable source of information on food quality and safety. A customer complaint procedure covers (i) written instructions to deal with complaints, (ii) authority to decide on the extent of the investigation and subsequent corrective action, (iii) documented recall procedure, (iv) process for investigating and responding to the consumer within the shortest possible time, (v) procedure to prevent a recurrence, if the complaint is justified, (vi) regular reviews of complaint reports for trends or any specific process requiring attention. A customer complaint involves the customer, the organization, and sometimes the regulatory authority. The organization has to ensure that all complaints are resolved promptly to avoid possible harm to the consumer.

58.6.18 Audits, Reviews, and Recall Procedure

Audits and reviews are used to verify whether the GMP activities are working correctly as planned. Audits are systemic and independent examinations involving on-site observation, interviews with staff involved with operations and review of records. On-site observation includes the inspection of documents, processes, and records. Records of monitoring activities, corrective actions, audits and calibration of equipment are checked for compliance with the GMP plan.

Reviews are carried out regularly to ensure that activities of the GMP plan are still relevant. It gives the opportunity for the organization to make any changes in the manner in which food is prepared or handled, leading to continuous improvement.

An effective recall procedure ensures that harmful food products are not available for consumption. The organization should ensure that effective procedures are in place to deal with food safety hazards to enable the complete and rapid recall of any defective products from the market. When a product has been withdrawn because of a health hazard, the recall of other products made under similar conditions should be seriously considered. Recalled products should be held under quarantine until the manner of disposal has been established and the product disposed of. These are discussed in Chapter 57 on HACCP.

58.7 ISO 9000 STANDARD, GMP, AND HACCP SYSTEM

The ISO 9000 standard is a management system for controlling and improving the performance of the organization. On the other hand, GMP is a code of practice that controls all aspects of manufacture. ISO 9000 provides a management structure for the effective implementation of the GMP program. The revised FDA Medical Devices GMP [13] places considerable emphasis on quality management systems. Quality policy, quality objectives, and management commitment are all essential features of this program [14]. The current regulations were designed after the publication of the ISO 9000 series (1994 version) [15] and 17 clauses have been adopted by the Medical Devices GMP regulations. HACCP system is a food safety program that provides an effective structure to GMP by providing a system that identifies, evaluates, and controls hazards that are significant to the production of safe food. Prior to the development of HACCP plans, it is necessary to review the existing programs and verify that all good manufacturing practices are in place and effective. The GMP program ensures that HACCP plans focus specifically on critical control points for product safety. If a GMP program is not adequately implemented, the HACCP plan will be less effective in ensuring product safety. ISO 9000, GMP, and HACCP systems are thus complementary to each

other and together they contribute to the production of safe food while making a profit for the organization by minimizing waste and having effective systems.

58.8 BENEFITS OF GMP

GMP is a dynamic program that enables the organization to make and maintain quality improvements. The principles of GMP offer greater assurance of food safety and quality through broad-based prerequisite programs and emphasize prevention and control of processes throughout the food chain. They are designed to introduce on-line process controls that can quickly respond to potentially hazardous situations and provide continuous measures of quality that can uncover problems and fluctuations as they happen and before the product is released. Combined with an effective HACCP plan, the safety of the food being processed can be readily detected and corrected at critical locations before the product is completely processed and packaged. Because of the generic nature of GMP, it is flexible enough to be adapted to different industries. An effective GMP program can offer several benefits to the organization, and some of the benefits are summarized in Table 58.4.

58.9 APPLICATION OF GMP PRINCIPLES

A GMP program ensures favorable conditions for the production of safe food. In combination with a proper HACCP plan, a robust food safety program can be developed. The generic principles behind the management of GMP activities listed in Section 58.6 can be conveniently applied to all food processing operations. However, control of components, production and processes, packaging and labeling, and storage and distribution may require specific consideration depending on the nature of the food product. These are discussed in the following applications.

58.9.1 Production of Fresh Produce

The production of fresh produce involves growing, harvesting, packing, processing, and transporting to the facility. The FDA has published [16] eight guidelines to ensure the microbial food safety of fresh produce: (i) Prevention of microbial contamination of fresh produce is more important than corrective actions following contamination. (ii) Growers and packers should adopt good agricultural practices in areas over which they have control while not increasing risks to the food supply or the environment. (iii) Human or animal feces in the orchard are a major source of contamination. (iv) When water is used for agricultural purposes and processing, its quality must be considered in order to minimize the risk of contamination. (v) Use of manure or municipal biosolids should be closely monitored to minimize the potential for contamination. (vi) Personnel hygiene and sanitation practices in the processing areas play a critical role in minimizing the potential for microbial contamination. (vii) Growers and packers must adhere to all regulatory requirements. (viii) Define authorities and responsibilities of personnel involved in all operations from growing to transporting fresh produce.

TABLE 58.4
Benefits of GMP

	Benefit
1	Creates awareness of food quality and safety among the staff
2	Increased confidence in product safety
3	Provides a starting point for HACCP program
4	Recognition internationally
5	Prevents regulatory noncompliances by meeting regulatory requirements
6	Prevents expensive failures
7	Reduces customer complaints and recalls
8	Improves profits

58.9.2 Production of Unpasteurized Apple and Other Fruit Juices

A variety of pathogenic organisms have been known to cause illness as a result of consumption of juice [17]. The most likely source of contamination is from fruits coming into contact with animal feces, water, containers, workers, or contaminated processing equipment. Therefore, GMP principles have to be applied to all stages from harvesting to the delivery of finished goods.

58.9.2.1 Orchard Management

A means to exclude domestic and wild animals should be used to prevent access of animals to the orchard. In locations where bird roosting is a problem, a means to scare birds should be used. To reduce the risk further, it is advisable to avoid animal manure. Water used to dilute pesticides can be a source of contamination and therefore orchard owners should be aware of the water quality. Records of pesticide and fertilizer applications have to be maintained.

58.9.2.2 Harvesting Practices

Sound, ripe fruits should be picked in clean bins and transported directly to the storage facility, sorting station, or juice processing plant, as appropriate. Drop fruits or rotten fruits should not be used for further processing. All bins should be labeled to show the orchard location, picking date, and picking crew.

58.9.2.3 Transportation

The vehicles used for transportation should be kept clean, and care should be taken to avoid damage and contamination during transport. The vehicle must be inspected prior to loading to ensure cleanliness and suitability.

58.9.2.4 Fruit Storage

Ideally, fruits should be pressed as soon as possible to prevent an increase of pH, which would favor pathogen growth during storage. However, if fruits need to be stored, rapid cooling to

0°C will maintain fruits in good condition. During storage and subsequent operations, fruits should be gently handled to minimize physical damage.

58.9.2.5 Fruit Sorting and Cleaning

Inspection for sorting should be done in a clean, dry, well-lit environment, and decayed, damaged, or otherwise spoiled fruits should be removed to prevent contamination of juice.

Fruit cleaning is done by effective washing, brushing, and rinsing. Food-grade sanitizers can be used for washing, and sanitizer levels are monitored during use. Sanitizers are rinsed from fruits unless otherwise instructed by the manufacturer's instructions. The water used for washing and rinsing should be free from pathogens and maintained at least 5°C warmer than the temperature of fruits. Wash and rinse water should not be recycled, and records of water quality have to be maintained in the facility.

58.9.2.6 Fruit Inspection and Processing

Processing apples in cold storage should be kept between 0°C and 4°C, or at the recommended atmosphere and temperature for the variety. Pressing, filling, and sealing areas have to be enclosed to prevent access to pests. The processing areas should be separated from areas where fruits are sorted and washed. Appropriate filter cloth and tubing should be used in production, and tubing should be sanitized and cleaned before commencing the operations. Pomace residue is disposed of after each day's production.

58.9.3 Production of Fermented Dry and Semidry Sausage Products

Dry sausages are meat products with a pH of 5.3 or less that are chopped or ground and dried to remove 25–50% of the moisture. Semidry sausages are dried to remove 15% of the moisture. In their manufacture, special attention is paid to the microbiological condition of the products [18]. Testing requirements for *E. coli*, *Listeria* sp., *Salmonella* sp., and *S. aureus* are part of GMP.

58.9.3.1 Raw Material Treatment

Because of the potential microbiological contamination, a strict, comprehensive sanitation program is necessary. The control program begins at the source of raw products and the sanitization procedures must ensure that containers shipping raw product to the factory, including cartons, boxes, tankers, and trucks, are kept clean.

58.9.3.2 Processing

During slaughter and processing, product flow must prevent cross-contamination between raw and finished products. Possibilities of cross-connection, including human, equipment, water, air, or piping arrangements, should be investigated to eliminate potential cross-contamination. pH and time–temperature are essential controls for fermentation and direct acidulation, and these are maintained at levels that inhibit the growth of microorganisms. Separate wash areas have to be established for raw and ready-to-eat (RTE) products, and they should be located where clean RTE equipment does not cross raw meat areas of the plant.

58.9.3.3 Slicing and Packaging Equipment

Complete mechanical disassembly is required to allow thorough cleaning and prevent contaminants from accumulating. All food contact surfaces have to be cleaned and sanitized daily. During production and breaks, the moisture level of the environment needs to be carefully controlled. Protective covers on control panels, motors, equipment, and other food contact surfaces can be a source of contamination and should be cleaned regularly. Heat shrinking equipment, including exhaust ducts, should be cleaned and sanitized daily to avoid spreading contamination from water and steam to packaging lines.

58.9.4 Poultry Processing

Poultry processing includes the processing of poultry, poultry parts, and other edible materials from poultry that have not yet been treated any way to ensure their preservation [19]. They are chilled or frozen and intended for human consumption.

58.9.4.1 Inspection and Sorting

Different domesticated birds should be processed separately to protect against the risk of cross-contamination. All poultry should be subjected to antemortem and postmortem inspection as required by regulations or established protocols. All unfit poultry should be removed, segregated, and disposed of appropriately.

58.9.4.2 Washing and Other Preparations

After evisceration and inspection, carcasses are washed in clean water. Wash water should not be recycled. The water used during the preparation, handling, packing, and storing of poultry carcasses, poultry parts, and other edible material should be potable.

58.9.4.3 Preparation and Processing

All operations leading to the finished product and packing should be carried out in a timely manner to enable the rapid handling of consecutive operations, which would prevent contamination, deterioration, spoilage, or the development of infectious or toxigenic microorganisms. Bleeding and blood collection, scalding, plucking, and evisceration should be carried out under controlled conditions to prevent any contamination. Bleeding equipment, scalding tanks, plucking machines, and evisceration troughs should be maintained in good condition and cleaned regularly.

Special precautions must be taken with wax-dipped poultry so that set wax and removed feathers fall into suitable containers. Only clean wax should be used for wax dipping. Reclaimed wax should not be reused unless it has been heated to 80°C for at least 20 minutes. Before reuse, the wax is skimmed, washed, and filtered.

58.9.4.4 Cooling and Refrigeration Requirements

After preparation, carcasses are cooled rapidly to an internal body temperature of 4°C or less. When cutting takes place before cooling, it is done within one hour of slaughter. Immediately after cutting, the parts are stored at 4°C. When cutting is done after cooling, the internal body temperature is not allowed to exceed 10°C. Poultry carcasses, poultry parts, and other edible materials are stored in an environment that prevents deterioration and mold growth. They should be regularly inspected and used in strict rotation.

58.9.4.5 Ice-Pack Containers

The ice used in ice packing should be made from potable water and should be manufactured, handled, stored, and used so as to prevent contamination. When poultry carcasses are ice-packed in barrels or other containers, they are wrapped in plastic or other suitable material to protect against contamination. The barrels and containers should have holes to permit the water to drain out and must be covered. Wooden barrels or containers should not be used for this purpose because wood can be a source of contamination.

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Part VII

Issues and Technologies Related to Food Preservation

59 Pesticide Residues in Foods: Their Sources and Reduction

Mohidus Samad Khan and Nadira Mustari

CONTENTS

59.1 INTRODUCTION

Pesticides are the substances or mixture of substances used to prevent, destroy, or control pests that may cause harm during production, processing, storage, transport, or marketing of foods and other agricultural commodities [1, 2]. The key objectives are (i) to control pests, plant diseases, and growth of organisms that could harm human activities and tree structures; (ii) to improve yield and quality of crops; and (iii) to save production costs of agricultural products [3, 5]. Globally, pesticide production, distribution, and application is a billion-dollar industry, which also provides job opportunities to millions of people around the world [6, 7]. However, the effects of pesticides and pesticide residues could be nonselective to pests and other living organisms, and may contaminate water bodies, affect nontarget and beneficial organisms, and persist in the environment for years. Populations are at high risk if they are exposed to direct or indirect sources of pesticides and/or pesticide residues. The entry sources could be the food and water chain. Therefore, a proper risk–benefit analysis needs to be done before using any type of pesticide.

To ensure safe pesticide use, regulatory actions have been taken by regulatory and environmental protection agencies of different nations, and hazardous synthetic chemical pesticides are being replaced by safer "organic" chemicals, such as biopesticides, which pose lower or no risk to the environment and human health [8]. However, lack of efficacy, inconsistent field performance, and high production costs are the limiting factors for them to become popular alternative products.

In this chapter, the ingredients of pesticides, classification of pesticides, relevant health concerns for different pesticide families, and management of pesticide handling are discussed. In addition, different pesticide management techniques, such as reduction of pesticide residues in grains and foods, alternatives to conventional pesticides, and prospects of organic farming are also covered.

59.2 HISTORICAL BACKGROUND OF PESTICIDE

The history of using pesticides to control pests is over 4000 years old. It is reported that different elemental compounds, such as sulfur, mercury, lead, arsenic, and copper, have been used as pesticides to control insects and pests [9]. The scopes and applications of pesticides have increased over the centuries to ensure high yield and to meet the demand of defect-free food production [10]. Worldwide, synthetic chemicals have been extensively used for the last few decades to inhibit or control pests, to reduce yield losses, and to uphold high-quality products. In recent years, the market of alternative pesticides, such as biopesticides, is also growing to reduce pesticide-related health and environmental hazards.

Humans have developed mechanisms to control pests since the beginning of agriculture. The earliest pest control strategies were conducted by the Sumerians in 2500 B.C.; they used sulfur compounds to control insects and mites. Greeks and Romans used oil sprays, ash, sulfur ointments, and lime to protect crops [11]. The modern-day chemical pest control era began in the early 1930s. Dichlorodiphenyltrichloroethane (DDT) was discovered as an effective insecticide in 1934 by the Swiss chemist Paul Müller [12]. DDT made a revolution in the world. DDT was used in Switzerland to control potato beetles in 1939 and commercial production began in 1943. It was effectively used in tropical countries to combat the anopheles mosquito, which transmits malaria. In 1946, Switzerland reported the first case of pesticide resistance: houseflies were not being affected by DDT anymore [13]. During the 1950s and 1960s, there were numerous reports of DDT-resistant plagues. In the following years, new types of pesticides were developed [14]. During this time, new approaches such as *integrated pest management* were developed and these are being adopted throughout the world to reduce pesticide consumption and increase efficiency in the control of pests.

The first legislation providing federal authority for regulating pesticides was enacted in 1910 [15]; however, decades later, during the 1940s, manufacturers began to produce large amounts of synthetic pesticides and their use became widespread [16, 17]. Pesticide use has increased 50-fold since 1950 [18, 19]. Seventy-five percent of all pesticides in the world are used in developed countries; however, the use of pesticides in developing countries is also increasing [20, 21].

In the 1960s, it was discovered that DDT was preventing many fish-eating birds from reproducing, which was a serious threat to biodiversity. Rachel Carson wrote the best-selling book *Silent Spring* about biological magnification, which also created an awareness revolution by pointing to pesticides as the reason for the systematic poisoning of the ecosystem [17]. The agricultural use of DDT is now banned under the Stockholm Convention on Persistent Organic Pollutants, but it is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes.

59.3 PESTICIDE INGREDIENTS

Pesticide products contain both *active* and *inert* ingredients. An active ingredient prevents, destroys, repels, or mitigates a pest; it can also be a plant regulator, defoliant, desiccant, or nitrogen stabilizer. All other ingredients are called inert ingredients by federal law of the United States of America [22]; they are important for product performance and usability.

59.3.1 Active Ingredients

Active ingredients are the chemicals in a pesticide product that act to control the pests. Active ingredients must be identified by name and weight percentage on the label of pesticide products. All ingredients other than biological pesticides and antimicrobial pesticides are considered conventional active ingredients. On the other hand, antimicrobial active ingredients are the substances or mixtures of substances used to destroy or suppress the growth of harmful microorganisms (such as bacteria, viruses, or fungi) on inanimate objects and surfaces. Ingredients derived from certain natural materials are called biopesticide active ingredients.

59.3.2 Inert Ingredients

Inert ingredients are combined with active ingredients to make a pesticide product. Inert ingredients are chemicals, compounds, and other substances, including common food commodities (e.g., certain edible oils, spices, herbs) and some natural materials (e.g., beeswax, cellulose). Inert ingredients play key roles in pesticide effectiveness and product performance. Inert ingredients serve the following functions: (i) act as a solvent to help the active ingredient penetrate a plant's leaf surface; (ii) improve the ease of application by preventing caking or foaming; (iii) extend the shelf life of a product; (iv) improve safety for the applicator; (v) protect the pesticide from degradation; and (vi) protect the pesticide from sunlight exposure. Under U.S. law, manufacturers are not required to identify inert ingredients by name or percentage on product labels. In general, only the total percentage of all inert ingredients is required to be on the pesticide product label.

59.4 CLASSIFICATION OF PESTICIDES

Pesticides can be classified according to their chemical structures, origins, working principles, target molecules, and possible health effects [23–29]. Different types of pesticides are briefly discussed in the following sections [23–30].

59.4.1 Inorganic Pesticides

Inorganic pesticides include arsenic, copper, and mercury compounds. Inorganic pesticides have the ability to remain in the environment for extended periods of time. They are generally neurotoxins and even a single dose may cause permanent damage.

59.4.2 Natural Organic Pesticides

Natural organic pesticides are mainly plant extracts, such as nicotine and nicotinoid alkaloids from tobacco; rotenone from the roots of derris and cubé plants; and pyrethrum, a complex of chemicals extracted from *Chrysanthemum cinerariaefolium* [11]. Even if natural, many of these compounds are toxic to humans and other life forms; recently rotenone has been linked to nerve damage and Parkinson's disease [31].

59.4.3 Fumigants

Fumigants are generally small molecules, such as carbon tetrachloride, carbon disulfide, ethylene dichloride, and ethylene dibromide, that easily gasify and penetrate rapidly into some materials. They are used to sterilize soil and prevent degradation of stored grain. These compounds are highly hazardous for workers, and their use has been severely restricted or banned.

59.4.4 Chlorinated Hydrocarbons

Chlorinated hydrocarbons are synthetic organics containing chlorine. They may be persistent in the environment and are subjected to bioaccumulation. Many chlorinated hydrocarbon pesticides have been banned or restricted throughout the world; however, some are still actively used. Chlorinated hydrocarbon pesticides include DDT, chlordane, aldrin, paradichlorobenzene, 2,4-dichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid.

59.4.5 Organophosphates

Organophosphate pesticides are synthetic organics containing phosphorus complexes. These inhibit cholinesterase, an enzyme that regulates the peripheral nervous system. These are degraded easily, so their bioaccumulation is rare. Some examples are parathion, malathion, dichlorvos, dimethyldichlorovinylphosphate (DDVP), and tetraethylpyrophosphate (TEPP).

59.4.6 Carbamates

Carbamate pesticides are derivatives of carbamic acid; they act in the same way as organophosphates and have low bioaccumulation rates. Carbamate pesticides are generally toxic to bees. They include carbaryl, aldicarb, aminocarb, and carbofuran.

59.4.7 Microbial Agents/Biological Controls

Microbial agents are living organisms that control pests. Bacteria, viruses, and insects have been used as "natural" controls. They can act in four ways: as parasites of the pest, as predators, as pathogens, or as weed feeders [30]. Table 59.1 lists different groups of pesticides based on their purposes. Figures 59.1 and 59.2 present pesticide classifications based on pesticide origins and chemical structures, respectively.

TABLE 59.1
Classification of Pesticides Based on Their Purposes

Group of Pesticides	Purpose
Algaecides	Kill or prevent growth of algae
Antimicrobials	Kill microorganisms that produce disease
Attractants	Attract specific pests using natural insect chemicals called pheromones that confuse the mating behavior of insects
Avicides	Control pest birds
Biopesticides	Naturally occurring substances with pesticidal properties
Defoliants	Cause foliage to drop from a plant, typically to aid in the harvesting process
Desiccants	Aid in the drying process of plants or insects, usually for laboratory purposes; promote drying of living tissues, such as unwanted plant tops
Fumigants	Produce vapor or gases to control air or soilborne insects and diseases
Fungicides	Destroy fungi that infect plants, animals, or people
Herbicides	Kill weeds and other plants that are growing or competing with a desired species
Insect growth regulators (IGRs)	Accelerate or retard the rate of growth of insects
Insecticides	Control or eliminate insects that affect plants, animals, or people
Miticides (Acaricides)	Kill mites that live on plants, livestock, or people
Molluscicides	Kill snails and slugs
Nematicides	Kill nematodes, which are microscopic wormlike organisms that live in the soil and cause damage to food crops
Ovicides	Control insect eggs
Piscicides	Control pest fish
Plant growth regulators (PGRs)	Accelerate or retard the rate of growth of a plant; substances (excluding fertilizers or other plant nutrients) that alter the expected growth, flowering, or reproduction rate of plants
Predacides	Control vertebrate pests
Repellents	Repel pests such as mosquitoes, flies, ticks, and fleas
Rodenticides	Kill mice, rats, and other rodents

Sources: World Health Organization [1]; McNaught and Wilkinson [2]; Cooper and Dobson [3]; Pimentel et al. [4].

59.5 HEALTH EFFECTS OF PESTICIDES

Pesticides are designed to control pests, weeds, and other plant pathogens; however, their mode of action is often nonspecific due to the presence of heterogeneous chemicals [32]. Pesticides often kill or harm organisms other than pests, including humans. Because of the improper and irrational use of different types of pesticides, the environment as well as the food chain (e.g. vegetables and fruits) may get contaminated with these chemical substances [33]. Individuals can be exposed to pesticides or pesticide residues through the workplace or due to environmental contamination. Based on the level of toxicity and exposure, these residues can affect different parts of the human body. The hazard of using pesticides depends on the toxicity of the pesticide and the amount of exposure to the pesticide, and it is often illustrated with the following equation:

$$\text{Hazard} = \text{Toxicity} \quad \text{Exposure}$$

The toxicity of a pesticide is a measure of its capacity or ability to cause injury or illness. The toxicity of a pesticide is determined by subjecting test animals to varying dosages of the active ingredient and each of its formulated products. The harmful effects of different pesticide families are briefly discussed in the following sections.

59.5.1 Health Effects of Fungicides

The acute toxicity of fungicides to humans is generally considered as low. Fungicides can be irritating to the skin and eyes. Inhalation of spray mist or dust from these pesticides may cause throat irritation, sneezing, and coughing. Long-term exposures to low concentrations of fungicides can cause adverse health effects [34]. Most cases of human fungicide poisonings have been from the consumption of seed grain. Table 59.2 summarizes the signs and symptoms of acute exposures to commonly used fungicides [34].

59.5.2 Health Effects of Herbicides

In general, herbicides have low acute toxicity to humans. However, there are different herbicides that can be dermal irritants since these are often strong acids, amines, esters, and phenols. Inhalation of spray mist may cause coughing and a burning sensation in the nasal passages and chest. Prolonged inhalation may cause dizziness. Ingestion usually causes vomiting, a burning sensation in the stomach, diarrhea, and muscle twitching [26, 35]. Table 59.3 summarizes the signs and symptoms of acute exposures to commonly used herbicides [26, 35].

59.5.3 Health Effects of Insecticides

59.5.3.1 Organophosphate and Carbamate

Insecticides cause the highest number of pesticide poisonings in the United States. The most serious pesticide poisonings usually result from acute exposure to organophosphate and carbamate insecticides. Organophosphate insecticides include chlorpyrifos, diazinon, dimethoate, disulfoton, malathion, methyl parathion, and ethyl parathion. The carbamate compounds include carbaryl, carbofuran, methomyl, and oxamyl. Organophosphates and carbamates inhibit the enzyme cholinesterase, causing a disruption of the nervous system. All life forms with cholinesterase in their nervous system, such as insects, fish, birds, and humans and other mammals, can be poisoned by these chemicals. Organophosphate and carbamate insecticides inhibit the activity of cholinesterase, resulting in a buildup of acetylcholine in the body. An increase in

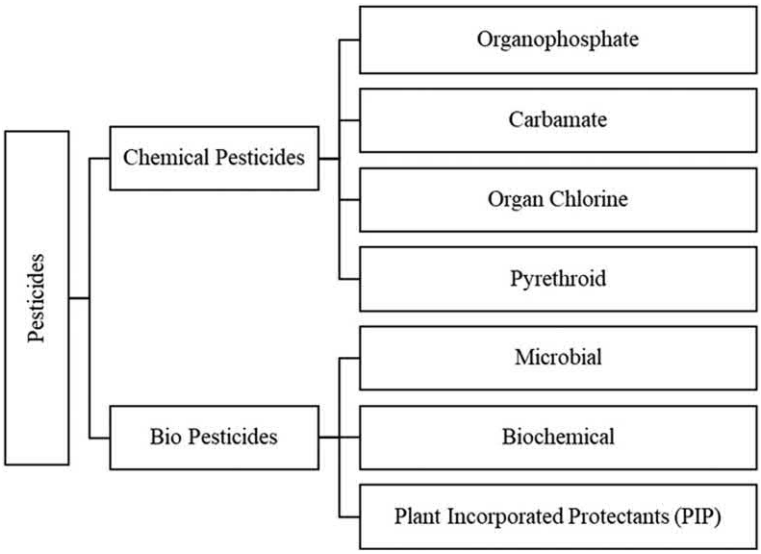

FIGURE 59.1 Classification of pesticides based on their origin. (From Rawtani et al. [28].)

acetylcholine results in the uncontrolled flow of nerve transmissions between nerve cells. The accumulation of acetylcholine causes the continual transmission of impulses across the synapses. The effects of organophosphate or carbamate poisoning can result in both systemic and topical symptoms. Table 59.4 summarizes the signs and symptoms from acute exposures to commonly used insecticides [26, 35, 36].

59.5.3.2 Organochlorine

Organochlorine insecticides are persistent organic pollutants (POPs), a class of chemicals that dissociate slowly in the environment and accumulate in the fatty tissues of animals [36]. Hence, POPs stay in the environment and food web long after being applied. Many POPs are endocrine-disrupting chemicals, which can create subtle toxic effects on the hormonal system of the animal body [37]. Endocrine-disrupting chemicals often mimic the natural hormones of the human body, disrupting the normal functions, and causing adverse health effects [38].

Among its wide variations, the mostly used organochlorine pesticides are DDT and its derivatives, such as hexachlorocyclohexane (HCH), aldrin, and dieldrin. These POPs are widely used in many countries because of their low cost and versatility against pests. Due to their potential bioaccumulation and biological effects, these POPs have been banned in different countries [39]; however, POPs are still available in the natural ecosystem [40]. Some of the names and related health effects of organochlorine pesticides are listed in Table 59.5 [41–48].

59.5.3.3 Pyrethroid Pesticides

Pyrethroid pesticides are potent neuropoisons, endocrine disruptors, and may cause paralysis [41]. Pyrethroids are a synthetic version of pyrethrin, a natural insecticide, and are more stable in sunlight than pyrethrins. Pyrethroid pesticides are popular insecticides as they can easily pass through the exoskeleton of the insect. Deltamethrin and cypermethrin are examples of pyrethroid pesticides [42].

59.5.3.4 Biopesticides

Biorational or biopesticides are considered relatively nontoxic to humans and environmentally safe. The U.S Environmental Protection Agency (EPA) defines biorationals as "certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals" [42]. The effect

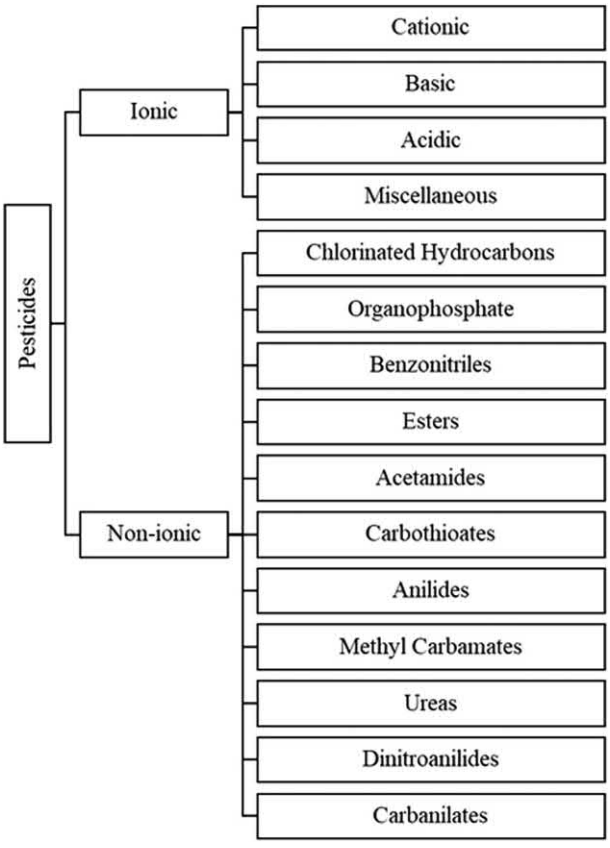

FIGURE 59.2 Classification of pesticides based on their chemical structure. (From Elzanaty et al. [29].)

TABLE 59.2
Signs and Symptoms of Acute Exposure for Several Fungicide Active Ingredients

Active Ingredient	Chemical Formula	CAS No.	Signs and Symptoms
Azoxystrobin	$C_{22}H_{17}N_3O_5$	131860-33-8	Irritating to skin, eyes, respiratory tract
Captan	C9H8Cl3NO2S	133-06-2	Irritating to skin, eyes, respiratory tract
Chlorothalonil	$C_8Cl_4N_2$	1897-45-6	Irritating to skin, mucous membranes of the eye, respiratory tract; allergic contact dermatitis
Copper compounds	—	—	Irritating to skin, eyes, respiratory tract; salts are corrosive to mucous membranes and cornea; metallic taste, nausea, vomiting, intestinal pain
Mancozeb	$C_4H_6N_2S_4Mn$ $C_4H_6N_2S_4Zn$	8018-01-7	Irritating to skin, eyes, respiratory tract
Maneb	$(C_4H_6MnN_2S_4)_n$	12427-38-2	Irritating to skin, eyes, respiratory tract; skin disease in occupationally exposed individuals
Pentachloronitrobenzene	$C_6Cl_5NO_2$	82-68-8	Allergic reactions
Sulfur	S	7704-34-9	Irritating to skin, eyes, respiratory tract; breath odor of rotten eggs; diarrhea; irritant dermatitis in occupationally exposed individuals
Thiram	$C_6H_{12}N_2S_4$	137-26-8	Irritating to skin, eyes, respiratory mucous membranes
Ziram	$Zn(R_2NCS_2)_2$	137-30-4	Irritating to skin, eyes, respiratory tract; prolonged inhalation causes neural and visual disturbances

Source: World Health Organization [1].

TABLE 59.3
Signs and Symptoms of Acute Exposure for Several Herbicide Active Ingredients

Active Ingredient	Chemical Formula	CAS No.	Signs and Symptoms
2,4-dichlorophenoxyacetic acid	$C_8H_6Cl_2O_3$	94-75-7	Irritating to skin, mucous membranes; vomiting, headache, diarrhea, confusion, bizarre or aggressive behavior; muscle weakness in occupationally exposed individuals
Acetochlor	$C_{14}H_{20}ClNO_2$	34256-82-1	Irritating to skin, eyes, respiratory tract
Atrazine	$C_8H_{14}ClN_5$	1912-24-9	Irritating to skin, eyes, respiratory tract; abdominal pain, diarrhea, vomiting; Eye irritation, irritation of mucous membranes, skin reactions
Dicamba	$C_8H_6Cl_2O_3$	1918-00-9	Irritating to skin, respiratory tract; loss of appetite (anorexia), vomiting, muscle weakness, slowed heart rate, shortness of breath; central nervous system effects
Glyphosate	$C_3H_8NO_5P$	1071-83-6	Irritating to skin, eyes, respiratory tract
Mecoprop	$C_{10}H_{11}ClO_3$	93-65-2	Irritating to skin, mucous membranes; vomiting, headache, diarrhea, confusion; bizarre or aggressive behavior; muscle weakness in occupationally exposed individuals
Metolachlor	$C_{15}H_{22}ClNO_2$	51218-45-2	Irritating to skin, eyes
Paraquat	$C_{12}H_{14}Cl_2N_2$	1910-42-5	Burning in mouth, throat, chest, upper abdomen; diarrhea; giddiness, headache, fever, lethargy; dry, cracked hands; ulceration of skin
Pendimethalin	$C_{13}H_{19}N_3O_4$	40487-42-1	Irritating to skin, eyes, respiratory tract
Propanil	$C_9H_9Cl_2NO$	709-98-8	Irritating to skin, eyes, respiratory tract

Sources: World Health Organization [1]; McNaught and Wilkinson [2].

of biopesticides depends on the interruption of natural growth processes of arthropods. These do not selectively attack any arthropod species, but generally have extremely low toxicity for vertebrates, including people. This group includes insect growth regulators (IGRs), chitin inhibitors, plant growth regulators, and chromosterilants.

59.5.3.5 Microbial Pesticides

Microbial pesticides kill arthropods, either by releasing toxins or by causing infections through microbial organisms. Two common microbial pesticides are *Bacillus thuringiensis* serotype *israelensis* (Bti) bacteria and *Bacillus sphaericus* (Bs) bacteria [43]. Products from these bacteria are used to kill mosquito larvae; Bti also kills black fly larvae. Most microbial pesticides are more selective than biochemical pesticides. The organisms used in microbial insecticides offer greater safety since these are nontoxic and nonpathogenic to wildlife, humans, and other organisms [44].

All pesticides have the potential to be harmful to humans, animals, other living organisms, and the environment if used incorrectly. The key to reducing health hazards when using

TABLE 59.4
Signs and Symptoms of Acute Exposure for Several Insecticide Active Ingredients

Active Ingredient	Chemical Formula	CAS No.	Signs and Symptoms
Acephate (organophosphate)	$C_4H_{10}NO_3PS$	30560-19-1	Headache, excessive salivation and tearing, muscle twitching, nausea, diarrhea; respiratory depression, seizures, loss of consciousness; pinpoint pupils
Aldicarb (N-methyl carbamate)	$C_7H_{14}N_2O_2S$	116-06-3	Malaise, muscle weakness, dizziness, sweating; headache, salivation, nausea, vomiting, abdominal pain, diarrhea; nervous system depression, pulmonary edema in serious cases
Carbaryl (N-methyl carbamate)	$C_{12}H_{11}NO_2$	63-25-2	Malaise, muscle weakness, dizziness, sweating; headache, salivation, nausea, vomiting, abdominal pain, diarrhea; nervous system depression, pulmonary edema in serious cases
Chlorpyrifos (organophosphate)	$C_9H_{11}Cl_3NO_3PS$	2921-88-2	Headache, excessive salivation and tearing, muscle twitching, nausea, diarrhea; respiratory depression, seizures, loss of consciousness; pinpoint pupils
Endosulfan (organochlorine)	$C_9H_6Cl_6O_3S$	115-29-7	Itching, burning, tingling of skin; headache, dizziness, nausea, vomiting, lack of coordination, tremor, mental confusion; seizures, respiratory depression, coma
Malathion (organophosphate)	$C_{10}H_{19}O_6PS_2$	121-75-5	Headache, excessive salivation and tearing, muscle twitching, nausea, diarrhea; respiratory depression, seizures, loss of consciousness; pinpoint pupils
Methyl parathion (organophosphate)	$(CH_3O)_2P(S)OC_6H_4NO_2$	298-00-0	Headache, excessive salivation and tearing, muscle twitching, nausea, diarrhea; respiratory depression, seizures, loss of consciousness; pinpoint pupils
Phosmet (organophosphate)	$C_{11}H_{12}NO_4PS_2$	732-11-6	Headache, excessive salivation and tearing, muscle twitching, nausea, diarrhea; respiratory depression, seizures, loss of consciousness; pinpoint pupils
Pyrethrins (natural origin)	—	—	Irritating to skin and upper respiratory tract; contact dermatitis and allergic reactions; asthma
Pyrethroids (synthetic pyrethrin)	—	—	Abnormal facial sensation, dizziness, salivation, headache, fatigue, vomiting, diarrhea; irritability to sounds or touch; seizures, numbness

Sources: World Health Organization [1]; McNaught and Wilkinson [2]; Cooper and Dobson [3].

pesticides is to limit the exposure by wearing personal protective equipment (PPE) and to use a low-toxicity pesticide when available. Reading the label and practicing safe work habits can minimize hazards from the use of pesticides.

59.6 MANAGEMENT OF PESTICIDE HANDLING AND USE

Because of the health and environmental hazards, worldwide pest management is facing economic and ecological challenges [45]. To overcome these challenges, regulatory actions have been taken by regulatory and environmental protection agencies of different nations. The manufacturer or the formulator along with the national authority should ensure proper labeling written in the local language(s) with warnings of possible hazards and comprehensive instructions for safe use. The users and producers should use personal protective equipment to prevent the risk of personal hazard [46]. In any pesticide poisoning, the first thing is to avoid further contamination, and it is important to ensure that the victim is breathing so that proper oxygen supply to the body can be maintained. Following this, medical assistance should be sought [47].

59.6.1 Pesticide Registration

Pesticide registration is a scientifically based, legal and administrative process, where a wide variety of effects associated with the use of a pesticide product and its potential effect on human health and the environment is assessed [48]. The registration is an important step in the management of pesticides as it enables authorities, primarily, to determine which pesticide products are permitted to use and for what purposes. Pesticide registration offers control over quality, usage rates, claims, labeling, packaging, and advertising of pesticides, thus ensuring that the best interest of end-users as well as the environment are well protected [1]. The registration process is restricted to the assumption that pesticides are only used for their intended function, and does not promote unreasonable effects either on human health or on the environment. Therefore, before commercial use, several tests are conducted that determine whether a pesticide has any potential to cause adverse effects on humans, wildlife, and aquatic ecosystem. Effects in any nontarget species may translate into ecosystem unbalance and food-web disruption that ultimately may affect human health and edible species.

Pesticide registration is a complex process and takes considerable time, resources, and expertise on the part of the registration authority, the pesticide manufacturing industry, and various public interest groups. An expanding series of tests based on improved technology is required to provide precise pesticide residue detections and toxicological assessments in response to public concern.

59.6.2 Human Exposure to Pesticides

Human exposure to pesticides may occur through occupational exposure in the case of agricultural workers in open

TABLE 59.5
Health Effect of Organochloride Pesticides

Name	Signs and Symptoms
BHC and its derivatives	Can harm the nervous system, β-BHC alters thyroid hormone levels and can affect brain development; may cause cancer [2], photosensitivity, permanent hair loss [1]
α- and ϒ- Chlordane	Inhalation or ingestion may cause toxic effects, such as headaches, depression, anxiety, poor balance, tremors, and mental confusion; may cause cancer in animals [3]
Endosulfan I and II and sulfate	Acutely neurotoxic; acute poisoning includes hyperactivity, tremors, convulsions, lack of coordination, staggering, breathing, nausea and vomiting, diarrhea, and, in severe cases, unconsciousness [4, 5]
DDD, DDE, DDT, and their derivatives	May cause pancreatic cancer, non-Hodgkin's lymphoma, breast cancer, leukemia, skin sensitization, allergic reaction and rash [6], affect nervous system, cause prickling sensation of the mouth, nausea, dizziness, confusion, headache, lethargy, incoordination, vomiting, fatigue, and tremors; causes reproductive problems in rats and birds [7]
Aldrin and dieldrin	Decreases the effectiveness of immune system; increases infant mortality; reduces reproductive success; causes cancer, birth, and kidney problems [8]
Endrin, endrin aldehyde, and endrin ketone	Swallowing large amounts may cause convulsions and lead to death within a few minutes or hours; less serious exposure results in headaches, dizziness, confusion, nervousness, nausea, vomiting [9]

fields and greenhouses, workers in the pesticide industry, and exterminators of house pests [49]. However, irrespective of whether the occupation involves the use of pesticides, the presence of such chemicals in the working environment constitutes potential occupational exposure. Evidently, workers who mix, load, transport, and apply formulated pesticides are normally considered to be the group that receives the greatest exposure because of the nature of their work and are therefore at the highest risk for possible acute intoxications [50]. In some situations, exposure to pesticides can occur from accidental spills of chemicals, leakages, or faulty spraying equipment. People living in close proximity to agricultural farms or places where pesticides are frequently used may be unintentionally exposed to pesticides or pesticide residues. The occupational exposure increases in the case of not paying attention to the instructions on how to use the pesticides and particularly when the user ignores basic safety guidelines on the use of personal protective equipment and fundamental hygiene practices such as washing hands after pesticide handling or before eating [51].

Several factors can affect exposure during pesticide handling. The form of formulation of pesticide products may affect the extent of exposure. Liquids are prone to splashing and occasionally spillage, resulting in direct or indirect skin contact. Solids may generate dust while being loaded into the application equipment, resulting in exposure to the face and the eyes. The type of packaging of pesticide products can also affect pesticide exposure; the size of cans, bottles, or other liquid containers may affect the potential for spillage and splashing [52]. Weather conditions at the time of application, such as air temperature and humidity, may affect the chemical volatility of the product, the perspiration rate of the human body, and the use of personal protective equipment [53]. Wind velocity increases evaporation of spray droplet, loss of pesticide from the target area, spray drift, and resultant exposure to the applicator. General hygiene behavior of workers and proper use of protective clothing during pesticide use also have a substantial impact on pesticide exposure. Furthermore, the frequency and duration of pesticide handling affect exposure. In particular, the exposure of an individual farmer that applies a pesticide once a year is lower than that of a commercial applicator that normally applies a pesticide for many consecutive days or weeks in a season [50].

59.6.3 Minimizing the Negative Impact of Pesticides

In recent days, there is a growing level of concern regarding pesticide use and their impacts on human health and environmental quality [54]. To address the increased concerns, policymakers and authorities are developing and implementing regulations aiming to protect both the environment and human health. Compliance with available standard guidelines for the safe use of pesticides and cautious measures during selling and storing pesticides can minimize most of the hazards related to pesticide exposure.

59.6.3.1 Measures during Selling

The manufacturer, the formulator, or the person responsible for labeling and registering the pesticides with the national authority should ensure proper labeling written in the local language(s) with comprehensive instructions for safe use and warning for possible hazards. The label should additionally specify the ingredients and also provide instructions for first aid in case of poisoning [55].

59.6.3.2 Measures during Applying Pesticides

Users should follow the instructions provided in the label of the container or packaging when using pesticides. The users and producers should use personal protective equipment, such as protective clothing made of butyl rubber, PVC, neoprene, laminated polyethylene fabrics, gloves, eye protectors, and masks to prevent the risk of personal hazard [46].

In the case of pesticides applied in agriculture, integrated crop management (ICM) includes guidelines to be followed by farmer unions to enforce actions for the production of safe agricultural products without contaminating the environment [56]. For pest control, ICM encourages the use of complementary methods of pest management to reduce animal pests or weed population below its economic injury level and to minimize pesticide impacts on the other components of the agroecosystem. Pest-resistant crops against insects and fungi, biological control, and other cultural or physical measures can be used as complementary methods. Pesticide applications on crops should include the following information [56]: (i) identify the appropriate pesticide for the specific pest attack on the plants or crops, (ii) use of a pesticide at the recommended

dose when a pest is found or it requires a precautionary treatment, (iii) optimization of pesticide use for economic saving through adjusted doses according to pest population density, and (iv) minimization of pesticide use by altering the cultivation system to lower the risk of pests.

To ensure the safety of agricultural workers, pesticide handlers and cultivators, the EPA activated the Worker Protection Standard (WPS) in 1995 [57]. The aim of the WPS regulation is to "minimize and mitigate pesticide exposure, and inform agricultural workers about the hazards of pesticides." It requires agricultural employers to notify workers about pesticide treatments and advances [57].

According to a study conducted by EPA, around 85% of the total daily exposure to airborne pesticides comes from breathing air inside the home [58]. Improper pesticide applications should be avoided at homes and offices. For any pest-related issue, alternative measures, such as temperature treatment, biological controls, and the least toxic baits should be applied. Spraying pesticides in lawns and gardens should be avoided [58].

59.6.3.3 Measures after Pesticide Poisoning

The chemicals of pesticides may injure humans in many ways. It is therefore important to take appropriate measures if pesticide poisoning or exposure occurs beyond the permitted limits. In the case of any pesticide poisoning, the following steps may be followed [47, 59–61]. The first step is to avoid further contamination and to ensure that the victim is breathing. There is a good chance of recovery if proper oxygen supply to the body can be maintained. Following this, medical assistance should be sought [47].

If someone swallows pesticides, the victim should be treated immediately. First, the label of pesticides has to be identified. There are two ways to help a victim who has swallowed poison: either (i) inducing vomiting or (ii) diluting the poison by having the victim drink milk or water [59]. Inducing vomiting is the quickest way to get the pesticides out of the stomach, however, it may not be effective in certain cases like when the victim is unconscious, and when the pesticide is highly corrosive or a highly concentrated petroleum product.

If the skin is directly exposed to pesticides, it is advised to wash off the pesticides immediately to prevent further exposure, followed by drenching the skin with soap and water carefully. In the case of chemical burning, cold running water should be used to wash the skin. The affected area then needs to be loosely covered with clean soft cloths. Further treatment should be carried out based on medical advisory [60].

In the case of eye injury, eyes should be washed with clean water immediately for around 15 min since the eye membrane can absorb pesticides faster than any other external part of the body. Eyelids should be kept open while washing with a gentle stream of water. Using any kind of drugs or eye drops are prohibited [47, 61].

The victim needs to take fresh air immediately after inhaling pesticides. An artificial respirator should be used while shifting the victim, and it is more important when the victim suffers from breathlessness. The victim should be kept as quiet as possible. Tight clothing should be loosened. If the victim is becoming unconscious, he or she should be protected from falling and his chin should be pulled forward to ensure proper airflow [47, 60].

59.7 METHODS OF PESTICIDE RESIDUE REDUCTION

Pesticides are used indiscriminately and excessively throughout the globe, and these residues remain in the food materials, water, fruits, vegetables, and total diet [62, 63]. These pesticide residues enter into the human body by consumption of the foods contaminated by pesticides, which leads to chronic disorders. Thus, the removal of these residues from food commodities utilizing different processing methods is very essential. Different household preparations, such as peeling, bleaching, washing plus cooking, parboiling, and saltwater washing, play an important role in the reduction of pesticide residues in foods [64]. Most food processing techniques help to reduce or eliminate pesticide residues on the surface or inside the food [65]. Table 59.6 summarizes common food processing techniques of processed foods [66–70].

TABLE 59.6
Methods of Pesticide Residues Reduction

Types of Pesticide	Food	Removal Mechanism	Reference
Endosulfan	Bitter gourds	Washing (30 s)	World Health Organization [1]
Dichlorvos	Soybeans	Washing (twice)	McNaught and Wilkinson [2]
Phosalone	Golden apple	Washing	Cooper and Dobson [3]
HCB	Tomatoes	Washing (vinegar)	Pimentel et al. [4]
p,p-DDT		Washing (10% NaCl solution)	
Dimethoate		Tap water washing	
Endosulfan	Bitter gourds	Peeling	World Health Organization [1]
Fenthion	Mango		
Dimethoate			
Malathion	Rough rice	Parboiling	Amir et al. [6]
Endosulfan	Bitter gourds	Cooking (10 min open cooking, 10 min steam cooking)	World Health Organization [1]

Each food processing operation collectively reduces the concentration of pesticides present in food commodities [71]. Almost all loose surface residues and polar chemicals are eliminated by washing. Preparatory steps like peeling and trimming remove the residues from outer portions. Various thermal processing treatments, such as pasteurization, blanching, boiling, cooking, steaming, canning, and scrambling, have been found valuable in the degradation of various pesticides depending upon the type of pesticide and length of treatment. A significant portion of nonpersistent chemicals is hydrolyzed and bleached out by hot water washing. Nonpolar chemicals (chlorinated hydrocarbons) are grimly detained in the waxy layers of fruits and vegetables. Peeling of fruits and vegetables completely removes the pesticide residues accumulated in waxy layers, however this process reduces the beneficial phytochemicals in fruits and vegetables [65]. Different methods to reduce or eliminate pesticide residues in food items are briefly discussed in the following sections.

59.7.1 Reduction by Washing

Washing is the most common form of food processing and is a preliminary step in both household and commercial preparation. Loosely held residues of several pesticides are removed during washing with reasonable efficiency [72]. Researchers reported that washing rice grains with water removed approximately 60% of the chlorpyrifos residues [73]. Washing with 2% saltwater demonstrated a very good effect in the removal of the residues below maximum residue limits (MRL)[74].

Chlorinated water and ozonated water are often used for the disinfection of fruits and vegetables. Scientists have demonstrated that washing using chlorinated water and ozonated water reduces pesticide residues, such as azinphos-methyl, captan, and formetanate hydrochloride, on fresh apples and in processed apple products [75, 76]. Acidic solutions (5–10% solution of acetic acid/citric acid/ascorbic acid/H_2O_2) were found to be more effective than other washing solutions to extract organochlorine pesticides from potatoes [77]. It was reported that washing potatoes with tap water or aqueous solutions of acetic acid and/or NaCl followed by blanching or frying of potatoes removed most organochlorine and organophosphorus residues [78]. Washing with soap and acetic acid solutions was found to remove profenofos residues in aubergines; a high percentage of profenofos residues were removed from peppers after washing using acetic acid solution, potassium permanganate, and tap water [79]. Researchers have demonstrated that washing grapes with tap water, tamarind solution (2%), salt solution (2%), baking soda, lemon water wash, or vinegar can effectively reduce organophosphorus residues up to 75% [80].

59.7.2 Reduction by Cooking Process

The cooking process can significantly reduce pesticide residues in food items. To demonstrate cooking effects to reduce pesticide residues, researchers randomly analyzed selected fruits for the initial deposit of pesticides. Each lot of pesticide-treated sample was subjected to different decontamination methods, namely, washing under running tap water, 2% salt solution, direct cooking, or dipping in 2% salt solution and cooking, and then analyzed for final remaining residues after treatment using a validated QuEChERS (quick, easy, cheap, effective, rugged, and safe) method utilizing GC-ECD (gas chromatography–electron capture detector), FPD (flame photometric detector), and GC-MS (gas chromatography–mass spectrometry) [74]. The cumulative effect of all four household processes caused substantial reduction in residues up to 95%. However, cooking in a pressure cooker for 5 minutes reduced pesticides 30–93% [74]. Washing with 2% saltwater followed by cooking demonstrated that the residues were reduced 98–100%; in addition, fat-soluble and water-soluble pesticides can be removed using this process.

59.7.3 Reduction Using Food-Grade Coatings As Processing Aids

Coating hams with vegetable oils or hot lard is a common practice in Spain to control mite infestations in dry-cured ham [81]. Some vegetable oils (including coconut, sunflower, groundnut palm, corn, and sesame) are used to protect cured fish from pests and to give fish a more attractive appearance [82]. A list of animal and vegetable oils (e.g., soybean oil, canola oil, corn oil, olive oil, mineral oil, and lard), sorbic salts (sodium/potassium/calcium sorbate), propionic salts (sodium/potassium/calcium propionate), iodide salts (sodium/potassium/calcium iodide), citrate salts (sodium/potassium/calcium citrate), short-chain alcohols (e.g., 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol), organic acids (e.g., maleic acid, citric acid, 3,3-thiodipropionic acid), butylated hydroxyanisole (BHT), and butylated hydroxytoluene (BHA) have been tested as dippings on dry-cured ham cubes [83]. Among all the tested substances, 100% lard, 50% propylene glycol (1,2-propanediol), and 10% butylated hydroxytoluene (BHT) were found highly effective at controlling mite reproduction under laboratory conditions [83].

59.7.4 Reduction of Certain Pesticides Due to Their Low Solubility

Tea is the most commonly consumed beverage in the world. It is prepared after infusing processed black tea in hot water. During the process of brewing, along with flavor and aroma, the residues of plant protection chemicals may also be transferred into the tea brew or infusion. The leaching of certain pesticides, such as ethion, endosulfan, dicofol, chlorpyrifos, deltamethrin, hexaconazole, fenpropathrin, propargite, quinalphos, and lambdacyhalothrin, from powdered black tea into the brew was studied. The rate of transfer of the pesticide residue from black tea to the hot brew was largely influenced by physicochemical parameters, such as water solubility and the octanol–water partition coefficient. Tea brews prepared from untreated black tea samples were fortified with standard

solutions of the respective pesticides, extracted, and analyzed using GC (gas chromatography) and HPLC (high-performance liquid chromatography) by following standardized methods. Results revealed that the rate of leaching of residues of these pesticides into the tea brew was low due to their low solubility in aqueous medium and high octanol–water partitioning coefficients [84].

59.7.5 Reduction by Organic Agricultural System

Researchers have demonstrated that organic foods contain a lesser amount of pesticide residues. In line with several published literature reviews, the French Agency for Food Safety (AFSSA) performed an up-to-date exhaustive and critical evaluation of the nutritional and sanitary quality of organic food [85] and reported that (i) organic plant products contain more dry matter and minerals (Fe, Mg), and contain more antioxidant micronutrients such as phenols and salicylic acid; (ii) organic animal products contain more polyunsaturated fatty acids; (iii) data on carbohydrate, protein and vitamin levels are insufficiently documented; (iv) 94–100% of organic food does not contain any pesticide residues; (v) organic vegetables contain far less nitrates, about 50% less; and (vi) organic cereals contain overall similar levels of mycotoxins as conventional ones. Therefore, organic agricultural systems have already proved able to produce food with high-quality standards and low pesticide residues [85].

59.7.6 Reduction by Other Techniques

Different processes, like bread making, milling, washing, and infusion, have also been found to cause considerable pesticide residue dissipation. Commercially produced bread is an important component of the every day diet in many countries. In one study, during the bread making process, bread was prepared from wheat flour spiked at different concentrations (1–4 ppm) with endosulfan, hexaconazole, propiaconazole, malathion, chlorpyrifos, and deltamethrin. It was observed that at the 4 ppm level of spiking the degradation of endosulfan, deltamethrin, malathion, propiaconazole, chlorpyrifos, and hexaconazole were 70%, 63%, 60%, 52%, 51%, and 46%, respectively. Yeast-mediated fermentation and baking at high temperature led to the degradation of the pesticides [86].

59.8 ALTERNATIVE PESTICIDES AND ORGANIC FARMING

Increased understanding and awareness of the adverse effects of pesticides on health and the environment is driving the demand for alternatives to pesticides. The alternative approaches consider pest problems within a broad context, which include the presence of natural enemies, the distribution of the pest population, active season of growth, and expected weather patterns [86–89]. Biopesticides can be a replacement for synthetic chemical pesticides. Biopesticides pose lower risk to the environment and human health [90, 91]. Many sustainable farms use integrated pest management (IPM) as an alternative to pesticides [92, 93]. The overall optimization of pesticide handling by following the existing regulations could contribute to the reduction of the adverse effects of pesticides on human health and the environment [94]. A mew major alternative mechanisms of pest management are described next.

59.8.1 Cultural Control

Cultural control is the deliberate alteration of the production system by targeting the pest itself through agronomic practices to avoid or reduce pest injuries to crops. These methods are utilized most frequently to control pest-related issues. Crop rotation, intercropping, sanitation, trap crops, and pest-resistant crop plants are a few examples of cultural control. These individual tactics of cultural control tend to be pest and crop specific [95].

59.8.1.1 Tactics to Prevent, Reduce, or Delay Pest Colonization of the Crop

The site selection, intercropping, trap crops, and altering the time of planting are key alternate approaches to prevent, reduce, or delay pest colonization of a crop. *Site selection* involves locating the crop field in such a manner that pests, from the site of the previous year's crop or from natural overwintering sites, cannot easily find their way there [96].

Intercropping is a practice that involves growing two or more crops in proximity. The most common goal of intercropping is to produce a greater yield on a given piece of land by making use of resources that would otherwise not be utilized by a single crop. Intercropping may concentrate the pest in a smaller, more manageable area so that it can be controlled by appropriate tactics.

Trap crops are grown as a control measure to lure pests away from the cash crop. Pests are either prevented from reaching the crop or concentrated in certain parts of the field away from the main crop. The principle of trap cropping relies on pest preference for certain plant species, cultivars, or a certain stage of crop development [97].

Plantation and harvest dates of some crops can be rearranged to reduce or to avoid potential pest damage [98]. Early planting ensures that seedlings have reached a nonsusceptible or tolerant stage when the pest appears. Planting needs to be done only after the emergence or immigration of the pests leaves the pests without hosts. Early harvest date may prevent pests from reaching damaging population densities or overwintering stage by harvest [95].

59.8.1.2 Tactics to Reduce Survival of Pests by Creating Adverse Biotic and Abiotic Conditions

Source reduction involves eliminating food, water, shelter, or other necessities that are important for pest survival [99]. Animal manure management is an effective sanitation practice used to prevent or to reduce fly-related issues in poultry and livestock operation. Crop rotation interrupts the normal life cycle of pests by placing them in a nonhost habitat. It is

highly effective to prevent different weeds, soilborne plant pathogens, and root-living arthropods. Sufficiently sparse plant and row spacing are important in preventing plant pathogens that usually require a certain moisture threshold to germinate and grow.

Tillage (soil-turning and residue-burying practices) and seedbed preparation reduce the number of soil-living pest stages [100]. Some forms of tillage can reduce pest populations indirectly by destroying weeds and volunteer crop plants in and around crop-production habitats.

59.8.1.3 Tactic to Reduce Injury Caused by Pests to Crop Plants

Plants resistant to pest attack are less preferred by pests as they adversely affect the pests' normal development and survival, or the plant may tolerate the damage without an economic loss in yield and/or quality [100]. Constitutive plant resistance is easy to use, cheap, and compatible with other pest management tactics. Induced resistance to herbivores and pathogens reduces plant exposure to the autotoxic environment of secondary compounds [101].

59.8.2 Physical and Mechanical Control

Physical and mechanical controls either kill insects and small rodents or make the environment unsuitable for them by attacking or setting up barriers. These methods are used for crop growing and household pest management [71, 103–105].

59.8.2.1 Barriers

Row covers, typically used for horticulture crops, are useful to keep insects away from plants. These are knitted tenuously with plastic or polyester fiber so that plants can still absorb sunshine and moisture from the air. Diatomaceous earth, made from fossilized and pulverized silica shells, is used to impair the protective cuticle layer of insects, such as ants. Consequently, the insects become vulnerable to becoming dry. As moisture diminishes the effectiveness of diatomaceous earth, it must be attributed at regular intervals.

59.8.2.2 Traps

Devices like flypaper or sticky boards, covered with a sticky and poisonous substance, are used to attract insects. Insects, attracted by those traps, land upon the surface and get glued. These traps are commonly used for capturing flies or leafhoppers.

59.8.2.3 Fire

Farmers consider fire to destroy insect breeding grounds. Fire burns the soil-top and kills insects present there. However, fire may kill beneficiary insects as well. Besides, some larvae can sustain below the surface of the soil.

59.8.2.4 Temperature

Different thermal conditions can be used to kill insects or to prevent their infestation. Cold storage prolongs the shelf life of agriproducts and retards the development of pests. Heat treatment is also effective in killing insect larvae in certain types of products.

59.8.2.5 Radiation

Gamma radiation kills all stages of pests in storage conditions. This is a common method that is employed to kill insects or insect larvae during export or import of large quantities of grains, fruits, and vegetables.

59.8.2.6 Ultrasonic Vibrations

Moths are often sensitive to bats' ultrasonic signals, quickly escaping from the area. Imitation of the bat's echolocation system helps in driving away the lepidopterous insect pests from the area.

59.8.3 Biological Alternatives

Biological alternatives can be used as a replacement for chemical pesticides to leave the ecosystem undisturbed. Biological alternative options can be broadly classified as (i) biological control, (ii) biopesticides, (iii) semiochemicals, and (iv) transgenic organisms [105]. Biological control, also known as biocontrol, is the use of natural enemies (predators, parasitoids, insects, or other arthropod species) to reduce the damage caused by pests. Biopesticides, also known as biological control, are based on pathogenic microorganisms or natural products that usually kill pests. The term biopesticide may also be used, more widely, to describe the application of biological agents, pathogens, predators, or parasitoids. In addition, botanicals, semiochemicals, and transgenic plants are sometimes described as biopesticides. According to the EPA, biopesticides are certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals.

59.8.3.1 Biological Control

Biological control involves the suppression of reproductive organisms through the actions of parasites, predators, or pathogens to restrict pest population at a lower average density [106]. There are three different approaches to biological control: *importation*, *augmentation*, and *conservation*.

Importation involves the enforcement of the natural enemies of a pest to a new locale the pest does not naturally inhabit. The process involves determination of pest-origin and consequently collection of appropriate natural enemies associated with the pest. Selected natural enemies are then passed through rigorous assessments, testing, and quarantine processes to ensure their appropriate use. Finally, the selected natural enemies are mass-produced and distributed [107].

Augmentation involves the supplemental release of natural enemies to boost the natural inhabitant population. In addition, the cropping system may also be modified to favor or augment these natural enemies, called habitat manipulation. During the critical time of the season, a small number of natural enemies to a pest are released, which is called inoculative release.

In the conservation method, biological control action is taken to enhance the effectiveness of existing natural enemies

to pests in the ecosystem. As natural enemies are already adapted to the habitat and target pest, their conservation becomes simple and cost-effective.

59.8.3.2 Biopesticides

As mentioned earlier, biopesticides are certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered as biopesticides.

59.8.3.3 Semiochemicals

Semiochemicals (from the Greek word *semeon*, meaning "signal") are chemical substances that mediate interactions between organisms. Semiochemicals are attributed to interspecific and intraspecific interactions, which are categorized as allelochemicals and pheromones, respectively [108].

59.8.3.4 Transgenic Organisms

Genes of one species can be modified or can be transplanted to another species. Organisms that have altered genomes are known as transgenic. Genetic modification with recombinant DNA techniques is the newest way of generating pest-resistant plants. The most successful commercial transgenic crops resistant to insects include cotton, maize, and potato[109].

59.9 CONCLUSIONS

Pesticides play an important role in producing reliable supplies of agricultural produce at affordable prices to consumers, improving the quality of produce, and ensuring high profits to farmers. Extensive use of pesticides has caused food and groundwater contaminations, and the destruction of beneficial insects. Pesticides and pesticide residues are an important source of injury and illness among farmers and farm workers. Pesticides have been linked to a number of health problems, including neurologic and endocrine (hormone) system disorders, birth defects, and cancer. To understand the dangers posed by pesticides and pesticide residues, it is important to consider the role of inert ingredients along with active ingredients of pesticides. Inert ingredients may have the potential to be anything but inert; some may have toxicities as high as some active ingredients.

Increased understanding and awareness of the adverse effects of pesticides on health and the environment is driving the demand for alternatives to pesticides. There are proven alternatives to pesticide use. These approaches consider pest problems within a broad context, which include the presence of natural enemies, the distribution of the pest population, active season of growth, and expected weather patterns. Many sustainable farms use integrated pest management (IPM) as an alternative to pesticides. IPM is a growing movement among farms of all sizes that incorporates a variety of techniques to eliminate pests while minimizing environmental damage. IPM strategies hold the key to reduce the deleterious impact of pesticides. In addition, suitable use of pesticides is necessary to protect the environment and, eventually, health hazards associated with it. Before choosing a pesticide, important aspects should be kept in consideration, such as proper identification of the pest, mode of action of the pesticide, and quality and persistency of the pesticide. Proper protection measures should be taken to avoid unnecessary pesticide exposure to the user as well as environment, and the pesticides should be stored in proper storage facilities. There are a number of treatments, such as washing with tap water, chlorinated water, ozonated water and/or acidic solutions; cooking or baking; using food-grade coating; and adopting an organic agricultural system that could be performed to reduce pesticide residue in foods.

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60 Biotechnology in Foods

Mohammad Shafiur Rahman

CONTENTS

60.1 INTRODUCTION

Biotechnology is one of the most important technologies affecting food production, processing, preservation, and security as well as nutrients contents. However, there are issues in favor of and against the use of biotechnology in food production. The World Health Organization (WHO) [1] defines genetically modified (GM) organisms as "organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination." This technology is often called "modern biotechnology" or "gene technology," "recombinant DNA technology," or "genetic engineering (GE)." In this technique, select genes are transferred from one organism into another, either from the same species or from nonrelated species. Foods produced using GM organisms are often referred to as GM foods. Biotechnology covers many contemporary agricultural and food manufacturing tools. In many instances, the use of the term GE over GM is considered more precise.

60.2 HISTORICAL BACKGROUND

The era of GE began in the early 1970s when California scientists discovered how to make recombinant DNA (rDNA) using restriction enzymes to cut and paste DNA, which gives a bacterium, plant, or animal a trait it does not possess naturally [2]. Transgenic crops were first planted commercially in 1995 and have increased rapidly since [2]. The first generation of genetically engineered foods started mostly with disease-insect and herbicide-resistant crops, such as glyphosate-resistant soybeans, insect-resistant corn and potatoes, and virus-resistant squash. The focus was to increase production as well as quality and safety (i.e., environmental and food) due to the reduction of chemicals. The second generation of biotechnology consists of crops to create healthier foods, for example, cereal grains with increased amounts of soluble and insoluble fibers, milk with improved calcium bioavailability, and vegetables with boosted levels of antioxidants [2]. The major concerns of biotechnology are the safety of transgenic crops for human consumption and the environment or ecology. Thus, it is important to know the proper and realistic risk-benefits of biotechnology.

60.3 ENHANCED GLOBAL FOOD SECURITY

A world without hunger is possible if food production is increased sustainably, and food safety is ensured with proper nutrition and distribution [3]. Food security demands sound supply of foods as well as safe and wholesome foods. GM foods could possibly meet the world's future food needs for the growing population by increasing production. This could be achieved by adapting diverse environments and tolerance to stresses. GM technology could provide food security by enhancing genetic diversity, for example, banana production. GM foods may contain nutritious foods, such as golden rice.

60.3.1 Increased Food Production

GE showed great promise for increasing crop productivity, for example, improvement genes in tomato controlled plant architecture, flower production, and fruit size, thereby improving productivity [4]. Molecular breeding programs and genetic engineering using de novo domestication can exploit and boost the genetic diversity of wild plants. Zsogon et al. [5] edited six loci genes that are important for yield and productivity in present tomato crop lines through de novo domestication of wild tomato. The morphology was altered, together with the size, shape, number, and nutritional value of the fruits as compared with the widely cultivated tomato (i.e., threefold increase in fruit size, a tenfold increase in fruit

number, compact growth, and 500% improved in lycopene accumulation, which is noticeable through a deeper red coloring of the juice).

Pests are a major threat to agricultural food production. Around 40% of worldwide crop production is destroyed by pests and pathogens, and 13% due to insect attacks. Among crops, the loss due to pests varied from about 50% in wheat to more than 80% in cotton production. In the cases of soybean, wheat, and cotton, the estimated losses varied from 26% to 29%, and for maize, rice, and potatoes the losses are 31%, 37%, and 40%, respectively [6]. Currently, the control strategies against pests are mainly based on chemical treatments and biological control. Interestingly, major focuses of GM are given to the increased grain yield and crop productivity. The new genome-editing technologies could address major pest and disease problems, reduce the need for chemical pesticides, and make plants more resilient to climate stress [3].

The earliest applications of genetic engineering to agriculture have focused primarily on simplifying pest management in widely planted crops. One major category includes crops for diseases and insect resistance to prevent crop losses (e.g., insect-resistant corn and cotton). The second category encompasses herbicide-tolerant crops, which allow farmers to spray broad-spectrum herbicides over growing crops [7]. One of the most harmful tomato pests is the *Tuta absoluta* (Meyrick) (Lepidoptera: Gelichiidae), and without appropriate management, it can cause production losses ranging from 80% to 100%. Hamza et al. [8] observed tomato defensive responses on this insect when a serine proteinase inhibitor (BTI-CMe) and a cysteine proteinase inhibitor (Hv-CPI2) from barley gens were used. They observed that the proteinase inhibitor 2 (Pin2) gene increased the production of glandular trichomes and altered the emission of volatile organic compounds.

Herbicide-tolerant (HT) GE soybean impaired plant root development and activity of microorganisms responsible for nitrogen fixation. The most substantial benefit of HT crop technology stems from its compatibility with no-till production systems. This benefits conservation efforts by less erosion and sedimentation of waterways, improved soil quality, less fuel and labor use, and better wildlife habitat.

60.3.2 Disease-Resistant GM Crops

In Bangladesh, there is a successful public–private partnership for the development of a commercial release of transgenic insect-resistant eggplant [3]. In Africa, a drought-tolerant variety of water-efficient maize is being developed with the intention to make this available royalty-free to smallholder farmers through African seed companies [9].

60.3.3 Increased Nutritional Value and Functionality

Biofortification is a promising, cost-effective, and sustainable technique to enhance nutritionally enhanced crops, and these could deliver micronutrients to a population with limited access to diverse diets or where micronutrient interventions are limited. Garg et al. [10] reviewed the success stories of biofortification research on different crops through breeding, agronomy, and genetic modification, and these approaches improved the lives of millions of people around the world. The GM approach can be used for the simultaneous incorporation of genes involved in the enhancement of micronutrient concentration, their bioavailability, and reduction in antinutrient concentration in plants. In addition, GM modifications can be targeted to redistribute micronutrients between tissues, enhance the micronutrient concentration in the edible portions of commercial crops, increase the efficiency of biochemical pathways in edible tissues, or even the reconstruction of selected pathways [11–13]. Initially, transgenic biofortified crops involved substantial amount of time, effort, and research investment and development. However, in the long run it is a cost-effective and sustainable approach, unlike nutrition-based organizational and agronomic biofortification programs [14, 15].

Modern biotechnology tools are also being used to develop these more nutritious crops. A lack of dietary diversity directly increases the risk of nutrient deficiencies. There is no single solution to the complex problem. The International Life Sciences Institute (ILSI) [16] reviewed five case-study crops with improved nutritional value. The first one is the "double-embryo maize," a variety being developed through modern biotechnology in which the grain contains two embryos, resulting in high protein and oil contents in maize. The second one is the "orange-fleshed sweet potato," a crop biofortified with β-carotene to control vitamin A deficiency. The third one is the "improved-protein sweet potato," with improved protein content, and it could be for the populations at risk for protein-energy malnutrition. The fourth one is "golden rice 2," with increased provitamin A carotenoid and β-carotene content. The fifth one is the "lysine-maize," with increased lysine content in order to simplify diet preparations. Zhu et al. [17] developed a high-efficiency vector system for transgene stacking and used it to engineer anthocyanin biosynthesis in rice endosperm. It was constructed by containing eight anthocyanin-related genes (i.e., two regulatory genes from maize and six structural genes from Coleus) driven by the endosperm-specific promoters, plus a selectable marker and a gene for marker excision. This transformation of rice generated a novel biofortified germplasm "purple endosperm rice" containing high anthocyanin content and antioxidant activity in the endosperm. The "Cavendish" dessert banana was genetically modified and greatly enhanced provitamin A levels and named the "golden banana" [18]. Cruz-Rus et al. [19] reviewed the efforts of improving the L-ascorbic acid (i.e., vitamin C) content in crop species using GM transformation, quantitative trait loci, and association-mapping-based approaches.

The transgenic approach can be a valid alternative for the development of biofortified crops, and it relies on the access to the unlimited genetic pool for the transfer and expression of desirable genes from one plant species to another. It is independent of their evolutionary and taxonomic status. Furthermore, transgenic approaches remain the only feasible

option to fortify these crops when a particular micronutrient does not naturally exist in crops. Garg et al. [10] pointed out that besides the challenges of biofortified crops, there is a bright future to address the malnutrition challenge thru biofortification. Garg et al. [10] presented the utilization of different genes for biofortification by transgenic means (Figure 60.1). They found that large numbers of genes have been utilized for crop biofortification. The transgenic-based approach has the advantage that once a useful gene discovered, it can be utilized for targeting multiple crops, including important genes like phytoene synthase. They summarized the enhanced crop nutritional level considering different micronutrients, such as vitamins, minerals, essential amino acids, and essential fatty acids. Most of the crops are in the research stage and very few are now released.

60.3.4 Flavor Enhancement

The release of the tomato reference genome when resequenced, considering hundreds of diverse cultivated and wild tomato accessions, showed that genomic changes through the history of tomato breeding [20]. Modern tomato breeding is primarily focused on yield, shelf life, and resistance to biotic and abiotic stresses [21]. Organoleptic and aroma quality traits are commonly ignored, resulting in a decline of flavor-associated volatiles [22]. The narrow genetic diversity of modern tomatoes limits their diversified improvement potential. Gao et al. [20] presented the tomato pan-genome constructed using genome sequences of 725 phylogenetically and geographically representative accessions. This revealed 4873 genes absent from the reference genome. They identified a rare allele in the TomLoxC promoter and observed that transgenic plants revealed a role of TomLoxC in apocarotenoid production, which contributed to desirable tomato flavor.

Freitas et al. [23] reviewed the marine biotechnology advances toward the production of new functional foods and ingredients, such as enzymes, chitin and chitosan, different types of proteins, bioactive peptides, amino acids, omega-3 fatty acids, pigments, phenolic compounds, and polysaccharides. Cruz-Hernandez and Paredes-Lopez [24] reviewed the applications of GM for ripening fruits since it is important to the development of color, aroma, flavor, and texture. These improvements could be attractive to potential consumers.

60.4 POLICY MAKING

In 1992, the US Food and Drug Administration (FDA) adopted a regulatory policy that specified that foods produced through genetic engineering techniques or containing GE substances substantially similar in "structure, function, and composition" to substances already in the food supply (proteins, carbohydrates, fats, and oils) were to be considered

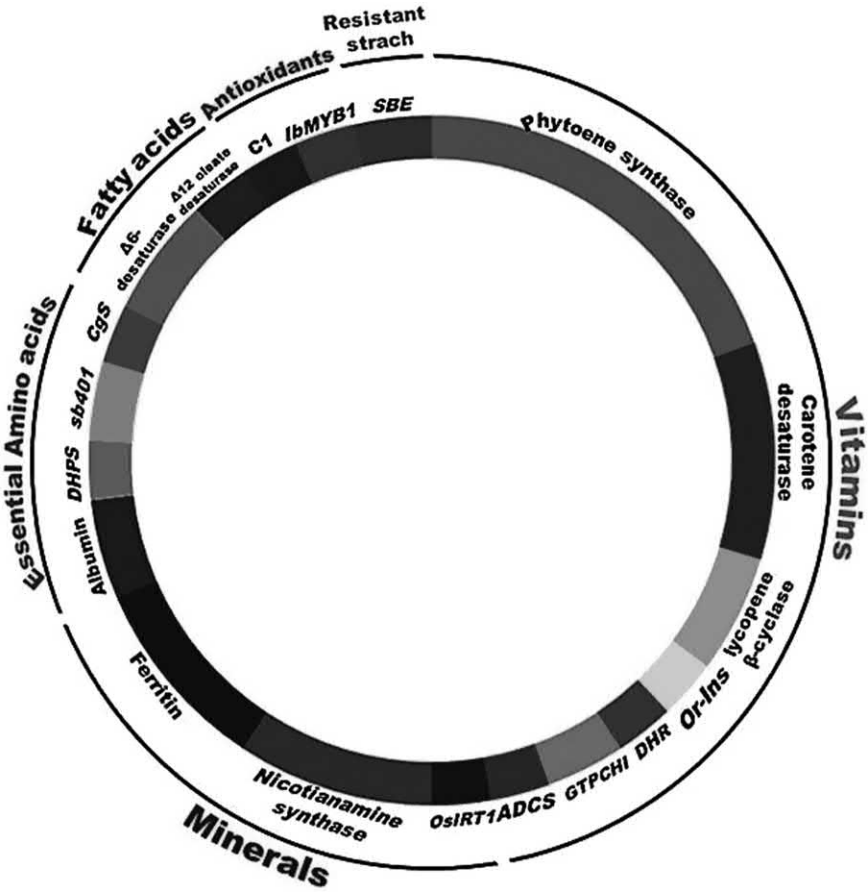

FIGURE 60.1 Utilization of different genes for biofortification by transgenic means. (From Garg et al. 2018 [10].)

"generally recognized as safe (GRAS)" [25]. This terminology was later changed to "substantially equivalent" (SE) [27]. If any food product considered SE is already in food supply (i.e., proteins, carbohydrates, fats, or oils), then it could be considered as GRAS. FDA deemed GM foods as GRAS or SE, and these are not required to undergo mandatory premarket approval [25, 27]. This policy was not accepted by many scientists. In January 2001, the FDA proposed modifications to its regulatory policy and called for a 120-day premarket notification [26].

The National Research Council (NRC) concluded that the US Department of Agriculture (USDA) needs to (i) more rigorously review GE crops before approving them for commercial use, (ii) more actively seek outside scientific peer review of crop applications and advice on changes in regulatory policy, (iii) more actively solicit public comment, and (iv) monitor transgenic crops more closely after their approval [28].

There is little history of safety regarding the structure, function, or composition of GM foods. The function of a food-producing organism could be altered, causing it to produce new allergens or increase the expression of allergenic proteins already present [2]. Scientific uncertainty is a concern owing to the novelty of health and environmental concerns due to the lack of established scientific methods [29]. The four central components necessary to deal with scientific uncertainty are (i) taking preventive action in the face of uncertainty, (ii) shifting the burden of proof to the proponents of an activity, (iii) exploring a wide range of alternatives to possibly harm actions, and (iv) increasing public participation in decision making [7].

In December 2002, the British Medical Association (BMA) supported precautionary principle toward GE foods. The precautionary principle handles at least two types of errors. A desire to minimize type II errors (false-negative) since the potential harm is greater if conclusions commit a type II error compared to a type I (false-positive), since recovery from most harm to ecosystems or human health involve large time lags and are sometimes irreversible. However, type I errors are usually limited to the short-term economic loss for the developers [30]. The second one is to take into account something that is inherent in all biological systems regarding uncertainty to the biological system. The precautionary principle is supported for four reasons [31]: (i) it benefits workers, (ii) it does not impose damaging costs on industry, (iii) it can stimulate technological innovation, and (iv) economic logic supports timely action to avoid substantial health and environmental costs. Criticisms of the precautionary principle include [32] (i) current regulatory processes are already precautionary, (ii) the precautionary principle is not scientifically sound because it advocates making decisions without adequate scientific justification, and (iii) if it were implemented, the precautionary principle would stifle innovation by requiring proof of safety before new technologies could be introduced [33].

A controversy surrounding GE foods stems from whether consumers have the right to know if their food has been bioengineered [2]. The requirements of GM labeling vary from country to country. The moral and ethical concerns depend on the history, traditions, experiences, beliefs, and values of a diverse citizenry. In general, the American national identity is generally not linked to food and food production, and the opposite is true in Europe. Thus, in Europe, GM foods could be a threat to their cultural identity. In some countries there is pressure on land, thus it is difficult to preserve wildlife. Europeans reported placing the highest confidence or trust in international organizations, such as the United Nations (UN) and WHO. In contrast, in the United States most support was reported for US government agencies, including the USDA and FDA. In many instances, local authorities could take quick actions for local outbreaks [7]. Recently, the USDA released "National Bioengineered Food Disclosure Standard" rules and regulations related to GE foods [34]. It is intended to provide a mandatory uniform national standard for disclosure of information to consumers and is effective from February 19, 2019.

The perception of science also varies. In Europe, science is considered a process of debate, thus ethical, social, and cultural factors are valued more highly. This produces lower confidence in the power of science and necessitates the adoption of the precautionary principle. Libertarian sentiment in the United States insists that science be halted only if there is sound scientific evidence of the negative aspects of GM foods [7]. Americans are most often addressed in their capacity as consumers; European often separate their role in society into citizens first and consumers second. Quality of citizen engagement also affects perception of GM foods.

Consumers would be more likely to buy bioengineered foods that taste better or fresher, protect against insect damage or require less use of pesticides, do not involve harm to animals, and do not involve the transfer of animal genes into plants. The lack of consumer acceptance of GE foods and crops is attributed to the public ignorance or misunderstanding of science, stems from the lack of trust between the public and other sectors of society, reports of trusted information from the industry, and skepticism of the government's ability to regulate GE technology [2]. Consumers' education and trust is very important and this could be achieved through labeling and disclosure, as well as exchange of knowledge. One-way exchange of knowledge to the consumers is perceived as passive reception, where only authority provides rules without allowing any feedback from the consumers [2].

More than 30 years of experience with GM crops shows that negative public attitudes in Europe can have a considerable effect on public perceptions and policy in developing countries [35]. The European Court of Justice ruled to regulate GE crops in the same way as GM, and this could stifle international progress in applying genome-editing technologies for crop improvement [36]. The rulings by the United States and Japan on the relaxation of regulations toward genome-edited crops are expected to set the ground for a new paradigm for leading to more efficient regulation internationally [3, 37]. A lower degree of restrictive regulations of genome-edited crops in the European Union could send a positive signal to the developing countries for GM technologies for food security [3]. Still, there is limited public acceptance and adoption of

GM technologies due to the lack of technical, regulatory, and communication capacities to handle transgenic GM technologies locally [38].

The societal risks depend on the public assimilating information from the scientific community. McFadden and Lusk [39] used Bayesian decision theory to explain risk factors of GM foods and global warming. They studied the effects of prior beliefs on the assimilation of scientific information related to GE by the public. Bayesian decision theory assumes that people update and incorporate a belief by allocating weights to a prior belief and new information to form a posterior belief. Information processing does not always conform to Bayesian decision theory and posterior beliefs may converge or diverge by new information. This disconnect implies that the public could place greater weight on other types of nonscientific information with heuristics and cognitive biases. For example, consensus by the scientific community may not be the same since the assimilation process of knowledge is different. It was observed that the extent to which new adapted information accepted depends on the extent to which it conforms to prior beliefs (i.e., factors), such as misinterpreting information, illusionary correlations, selectively scrutinizing information, information-processing problems, knowledge, political affiliation, and cognitive function.

In order to achieve sustainable progress, continued efforts, learning lessons from past, and strategy is necessary to facilitate the use and adoption of genome-edited crops and GE technology. Strategy should be based on transparent communication, and training of researchers and other stakeholders. The development of the evidence-based regulation, communications to build awareness, careful marketing strategy, capitalizing success stories, private–public partnerships, and capacity building (i.e. training, incentives, and facilities) should be emphasized [38].

After analyzing the safety issue, Nicolia et al. [40] pointed out that an improvement in the efficacy of scientific communication could have a significant impact on the future of GE. They concluded that their reviewed collection of scientific records be available to researchers, communicators, and teachers at all levels to help create an informed and balanced public perception on the important issue of GE foods. Implementation of rules related to GM is very challenging [41].

60.5 RISK–BENEFIT ANALYSIS

Participatory approaches are commonly used in risk analysis and risk decision-making. In 1996, NRC proposed three rationales for the broad participation in risk characterization: normative (i.e., participation of citizens), substantive (i.e., participation of people with diverse experience), and instrumental (i.e., participation of governments and international bodies) [42]. It is also emphasized for public–private partnerships and "Safety First Initiative" approaches. GM poses a threat to the values of pluralism or diversity. Intellectual property rights could allow limited access to all users, and thus the law should allow the rights of farmers, the community, and plant breeders.

ILSI [16] provided ten recommendations to assess the safety of GM crops. These are

(i) The new crop's safety assessment should start with an appropriate comparator crop that has a history of safe use.
(ii) It is necessary to develop data on a case-by-case basis, i.e., data for each crop must be developed individually.
(iii) The safety of any novel protein(s) introduced into a crop needs to be assessed carefully.
(iv) Compositional analysis of crops should include known toxicants and antinutritional compounds considering specific metabolites of the targeted metabolic pathway.
(v) The appropriate phenotypic properties need to be assessed, and unintended and unexplained differences need to be identified.
(vi) Laboratory animal studies need to be performed in addition to other safety assessments, and this will provide added safety assurance.
(vii) If GM is used for livestock feed, it is important to demonstrate the expected nutritional benefits.
(viii) Premarket studies in humans should be appropriate on a case-by-case assessment.
(ix) Premarket assessment needs to be checked to identify the impact of nutrient intake by the consumers when the diet is changed with GM crops.
(x) It may be useful to make a risk–benefit analysis of the removal of risk of harm caused by nutritional deficiencies.

All these issues will provide the relevant data required for a meaningful risk–benefit analysis.

Seralini et al. [43] studied the long-term toxicity (2 years) of herbicide (glyphosate: 0.1 ppb in water) used on maize and GM maize using rats. Glyphosate consumption in water above the authorized limits may provoke hepatic and kidney failure, according to the United States Environmental Protection Authority (US EPA). This study showed that concentrations well below officially set safety limits induced severe hormone-dependent mammary, and hepatic and kidney disturbances. Similarly, GM maize gave comparable pathologies that may be linked to abnormal or unbalanced phenolic acids, metabolites, or related compounds. In addition, other mutagenic and metabolic effects of the edible GM maize cannot be excluded, for example, the production of other potentially active compounds such as miRNAs [44] or leukotoxin diols [45]. This indicated that the use of toxic chemicals (i.e., herbicide) could be reduced, but similar toxicity could be developed by different new components in GM foods.

Nicolia et al. [40] reviewed the scientific literature on genetically engineered crop safety for the last 10 years. They built a classified and manageable list of scientific papers and analyzed the distribution and composition of the published

literature from 2002 to 2012. They tried to catch the scientific consensus from the selected original research papers, reviews, relevant opinions, and reports; and pointed that any significant hazards were directly connected with the use of genetically modified crops although the debate is still intense.

Cotter [46] highlighted in his report the unintended effects and potential risks related to GE in agriculture considering observations as reported in peer-reviewed scientific studies. He emphasized that significant research and data gaps in the unintended genetic mutations may impact human health and ecosystems. Many studies evidenced the intended changes that GE might achieve, while there is a complete lack of studies on the unexpected effects arising for the food and environmental safety. The "off-target" (i.e., changes to other genes that were not intended) effects are both a major challenge and a major concern. The off-target effects could unintentionally alter important genes, which can change in chemistry or protein production, and these dynamics and kinetics are important for food and environmental safety. In addition, any implication of the "on-target" effects (i.e., intended changes occur at the intended location, but with a different outcome than expected) needs to be carefully evaluated. For example, a small insertion or deletion of DNA within a gene, even if on target, could change the way a gene is read and processed into proteins in problematic ways causing toxicity. GE may be precise, but outcomes may not always be precise. Overall, regulatory oversight is essential to ensure that potential risks of GE organisms are considered prior to their entry into the environment or food chain.

Many countries in Africa and Asia are hesitant to promote the use of GM crops, largely because of erroneously perceived risks and fears of losing export markets to Europe [3]. The nature of the risk to the environment by GM will vary with the type of organism released. In one study, transgenic manipulation genes in Japanese medaka fish were selected and it was observed that body size reduced their reproductive fitness. It was estimated that the release of even one GE fish could lead to local extinction of the species within 37 generations. Another concern is that insects, which feed on cotton, corn, and other crops, may eventually develop resistance to the naturally occurring insect toxin *Bacillus thuringiensis* [2].

Lassoued et al. [47] provided an expert survey on the added potential benefits of GE or GM crops as compared to those developed by conventional breeding. The survey results revealed a consensus among experts on the enhanced agronomic performance and product quality of GE crops over alternatives. The majority of experts indicated that the regulations for health and safety, potential of export markets, consumers' willingness, and role of media play a major part in determining the development of GE and use in agriculture. They pointed out that further research is needed to gauge expert opinion for the success of GE technology. Recently, consumers of certain countries are more willing to consume GM-derived food [48] and success stories are available.

Moreau [49] studied the ecological risk analysis of GM salmon in relation to its management in the face of uncertainty. He reviewed the available empirical information on the potential ecological and genetic effects and discussed the underlying eco-evolutionary science. The data gaps and irreducible epistemic uncertainties limit the role of scientific inference in support of ecological risk management. The predictive uncertainties are pervasive in complex eco-evolutionary systems, and it is responsibility of those involved in the risk analysis process. These limitations need to be communicated timely, clearly, and cautiously.

60.6 CONCLUSION

Biotechnology could be one of the important technologies to address food security by increasing food production and nutrient content. There are issues in favor and against the use of biotechnology, although its application is progressing. Biotechnology (i.e., GM and GE) can increase crop productivity by affecting plant architecture, and creating insect-resistant, disease-resistant, and harsh-climate-resistant crops. In addition, biofortified crops can be developed for increased nutritional value, sensory quality, and health functionality. Rules and regulations are being developed and implemented to control the use of biotechnology, although lots of concerns and debates exist. Careful risk–benefit analysis needs to be conducted before applying biotechnology in food production.

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61 Food Laws, Regulations, and Standards

Rubaba Rahman and Mohammad Shafiur Rahman

CONTENTS

61.1 INTRODUCTION

The regulation of food safety has become more relevant than ever. In just 2018 alone, the world witnessed widespread adulteration of foods: strawberries in Australia were contaminated by small needles leading to a nation-wide trade recall of berries [1], a norovirus outbreak in the Winter Olympics in Korea impacted close to 200 people [2], and a listeria outbreak in South Africa led to over 180 deaths and around 1000 reported incidents [3]. It is such incidents that call for strong legislation and policies with respect to food safety to ensure consumer protection and public health. Indeed, the regulatory framework of food safety plays a crucial role in sheltering the public from economic fraud and preventing the sale of unsafe foods [4]. It is also widely found that inadequate laws relating to food safety result in foodborne illness outbreaks and affect the sale of foods, leading to serious economic consequences. McKeown et al. [5] studied the effects of food safety laws, consumer confidence, and foodborne illness outbreaks on the respective effects on food sales and found that consumer confidence in food safety resulted in losses in sales for many businesses. The increasing role of the media reporting such incidents further exacerbates public distrust. This chapter will accordingly first provide the reader with the principles underpinning legislation and policies concerning food safety.

It will then review how food safety is achieved through the current regulatory and legal framework, both internationally and locally, including in the United States, Canada, Dubai, Oman, Australia, and Europe. A global lens is necessary in an era of globalization and free trade. Alongside international and local laws, the chapter will also discuss how certifications and international standards further foster an environment of safe food production and supply. The chapter will conclude with future challenges that the current regulatory framework of food safety faces, including globalization, health threats, and technologies identified by the European Parliament's Committee on Environment, Public Health and Food Safety [6]. In this regard, using the Novel Food Regulation (NFR), de Boer and Bast [4] emphasized fostering mutual understanding and improving the use of science in regulatory acts. They highlighted that it is of utmost importance to bridge the gap between both fields of expertise.

61.2 ORIGINS OF FOOD LAWS AND REGULATIONS

The key to food safety is the strong and effective enforcement of laws [7]. By way of background, laws are normally a system of rules that are enacted by a governing body and are made up of legal obligations to do or refrain from doing something, whereas regulations are the process of monitoring and enforcing the rules. Guidelines flow from laws and regulations, which are advisory rather than binding [8]. Food law encompasses a wide range of legislation and accompanying regulations and guidelines that govern the way we cook, process, serve, distribute, and eat food. An effective legal framework aims to control the quality and safety of the food we eat, the training and expertise required of the people that provide food to the public, and the type of entity through which food can be served (i.e., a restaurant, a food truck, a food cart, etc.). Food laws affect every single one of us. They impact individuals and organizations, from the family who owns the convenience store down the street to the giant grocery conglomerate, and from the woman running the community kitchen to international food service companies. These laws govern supper clubs, farmer's markets, restaurants, grocery stores, and food festivals, to just name a few.

Food laws are mainly established based on risk and severity. Risk is the chance or probability that a given hazard will

occur, whereas severity is the degree of damage one hazard could cause. "Risk thermostat" is the balancing behavior and cultural filter of the propensity to take risks based on perceived danger, and its consequences could be rewards and accidents. Sperling [9] argued that ethics should play an important role in all three levels of risk analysis of food safety. He found that ethics encourage individuals to reflect on and make value judgments in the process of risk assessment.

61.3 STANDARDS AND CERTIFICATION

61.3.1 Standards

Maidana-Eletti [10] defines standards as being used in all areas of human activity to achieve certain characteristics of goods and their manufacture, thereby reducing risks, fostering trust, and enabling predictability in the market. Indeed, historically, consumers needed to trust the safety and quality of the food products they bought. They particularly required the products to be in accordance with their specifications or the "standards" they set. Before placing an order, the purchasing organization would normally make inquiries relating to the supplier's capability of supplying a product that will meet all its requirements. The purchaser would call for samples from potential suppliers and would carry out inspections and tests to determine whether the samples conformed to specifications. However, the actual shipments would still contain nonconforming products despite the samples having passed all the tests. Major buyers would therefore generally also send their technical experts to assess the quality control systems of the suppliers to ensure that the suppliers were capable of truly producing goods and/or services of consistent quality.

Evidently, the preassessment procedure of the suppliers' quality capabilities meant tremendous costs for buyers, particularly when overseas suppliers were involved. Multiple assessments by different purchasers would likewise be extremely expensive for the suppliers because of the extra labor and money required to prepare for each assessment. Another problem the suppliers faced were the subjective nature of each assessment. Experts from different purchasing organizations, drawing on their company's own experiences, would have different perceptions of an effective quality control system. As a result, suppliers would sometimes receive varying assessment reports from different purchasers for the same product and production system.

As identified by Keener et al. [11], substantial changes in the global economy, paired with increasing relationships between regulatory agencies internationally and a common goal to improve public health, have led to calls for international harmonization of food safety standards. The US Food and Drug Administration (FDA) reports that "such harmonization, potentially, enhances public health protection and improves government efficiencies by reducing both unwarranted contradictory regulatory requirements and redundant applications of similar requirements by multiple regulatory bodies" [11].

A universally accepted standard quality assurance system was deemed necessary to solve the aforementioned issues. It would serve as a reference or benchmark for the assessment of any supplier's quality system. Third-party assessors would also enforce a universal standard, reducing the need for purchasers to carry out individual evaluations and sparing suppliers the burden of multiple assessments.

It is for these reasons that the Codex Alimentarius Commission (CAC), a joint body of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), with 188 member countries and the European Union, introduced the Codex Alimentarius (Codex), or "Food Code." Codex encompasses international food standards, guidelines, and codes of practice contributing to the safety, quality, and fairness of the international food trade and protection of public health [12]. Since 1963, Codex has worked to foster harmonized international standards relating to food. Codex has published over 200 standards to date, including general standards for the labeling of prepackaged foods and standards for a variety of food products, from corned beef and canned crab meat to dates, bananas, and infant formulas [13].

Whether Codex is legally binding in nature has been hugely contested among legal academics, without any agreement being reached [10]. When the standards weren't adopted for local legislation, they remained nonbinding and were accordingly ignored for their lack of legal relevance [10]. However, the Sanitary and Phytosanitary (SPS) Agreements, which all members of the World Trade Organization (WTO) adhere to, adopted Codex, consequently upgrading Codex's legal status to semibinding [10]. The adoption of Codex by the SPS Agreements are in the following articles of the SPS Agreements.

> Art. 3.4: Members shall play a full part, within the limits of their resources, in the relevant international organizations and their subsidiary bodies, in particular the Codex Alimentarius Commission … to promote within these organizations the development and periodic review of standards, guidelines and recommendations with respect to all aspects of sanitary and phytosanitary measures.
>
> Art. 12.3: The committee shall maintain close contact with the relevant international organizations in the field of sanitary and phytosanitary protection, especially Codex Alimentarius Commission ... with the objective of securing the best available scientific and technical advice for the administration of this Agreement.

Alongside Codex, the Hazard Analysis Critical Control Point (HACCP) system and the ISO 22000 standard further regulate food quality internationally. HACCP aims to deal with possible risks for food safety [14]. ISO 22000 does not take a preventative approach, but rather a management and systems approach to implement food safety into an organization [14].

However, the rise of a variety of international standards has created more issues. Keener et al. [11] state that predominantly "rich, technologically advanced nations" are involved in the creation and discourse of standards, such as in the CAC. It is argued that poorer countries may struggle to adhere to these standards alleged to be more appropriate

for the wealthier nations [15], leading to a form of *techno-imperialism*, where rich nations impose their standards on the poorer countries, who have had limited to no input in the creation of the standards [11]. An international and collaborative approach to standardization can easily remedy this.

61.3.2 Certifications

Certification refers to the confirmation of certain characteristics of an object, person, or organization. This confirmation is often, but not always, provided by some form of external review, education, assessment, or audit. Accreditation is a specific organization's process of certification. One of the most common types of certification in modern society is professional certification, where a person is certified as being able to complete a job or task, usually by the passing of an examination.

In first-party certification, an individual or organization providing the good or service offers assurance that it meets certain claims. In second-party certification, an association to which the individual or organization belongs provides the assurance. Third-party certification involves an independent assessment declaring that specified requirements pertaining to a product, person, and process or management system have been met. In this respect, a notified body is a third-party, accredited body that is entitled by an accreditation body. Upon definition of standards and regulations, the accreditation body may allow a notified body to provide third-party certification and testing services.

61.3.3 Local Laws and Regulations

Alongside international regulations, local laws also govern food practices and trade. For example, the Food Safety Modernization Act, introduced in 2011, regulates food safety in the US. In 2013, it enacted two main rules to address food safety both domestically and when imported: "preventive controls for human food" and "standards for produce safety" [11]. In Canada, 14 statutes govern food safety, from the Food and Drugs Act to the Safe Food for Canadians Act, and similarly tackle issues relating to food safety and the importation/exportation of food [16]. In the Middle East, for example in Oman, Ministerial Decree No. 2 of 2010 issues regulations on food safety and handling, setting strong standards for hygiene [17]. In Australia and New Zealand, the Food Standards Australia New Zealand Act 1991 establishes a joint food safety standard system between the two countries. Legislation covering all stages of the food chain, termed the "farm to fork" approach [6], is imposed on each member state of the European Union. The European Parliament and the Council adopted Regulation (EC) No 178/2002, covering the basic principles of food law [18]. However, what happens when local legislation clashes between countries? For example, genetically modified foods are treated cautiously under European food laws, but considered safe under US, Argentinian, and Canadian laws [19] (see Figure 61.1, adopted from Lau and Lyon [20]).

Keener et al. [11] highlight that where such conflicts occur amongst local food laws, international agreements, like the aforementioned SPS Agreements, should assist. The SPS Agreements state that where countries impose strict standards, scientific justification must be demonstrated (Art. 2.2). The standards must be "based on a scientific risk assessment" (Art. 5.1). In this way, the Agreements encourage countries to base their food laws and regulations on international guidelines, sound scientific evidence, and public health, in a way that least detriments free trade [11, 19]. After litigation back in 2003 regarding genetically modified organisms (GMOs) in the World Trade Organization, it was found that Europe's stringent standards were not founded on the basis of risk assessment [21].

61.4 FUTURE CHALLENGES AND CONCLUSION

Pederson and Hernandez [6] reported to the European Parliament's Committee on Environment, Public Health and Food Safety on the future challenges of food safety law. They remain relevant and should be carefully considered by future policy makers. The challenges that Pederson and Hernandez flag, alongside other academics, are detailed in the following sections.

61.4.1 Globalization and Increasing Free Trade Agreements

With increasing global trade, the distribution of contaminated food can be extensive, making international standards and global cooperation essential (covered earlier). Menon [22] states that the number of free trade agreements is expected to increase in the future, which should foster international standardization.

61.4.2 Climate Change and Health Threats

Scientists warn that climate change not only means an increased average global temperature, but also comprises trends toward stronger storms, more rain, and prolonged dry periods [23]. Echoing Jaykus et al. [23], Pederson and Hernandez [6] found that such drastic climate changes are likely to affect food safety. Warmer climates augment hygiene risks when storing and distributing food. As such, climate change is expected to, and has actually been well documented in some places to, drastically impact food and waterborne diseases as well as zoonoses (diseases passed between animals and humans). For example, in Australia, notifications for *salmonellosis* increase with decreasing latitude, in other words, with increasing average temperature [24], which draws the connection between warmer climates and diseases. Miraglia et al. [25] found that with more extreme weather conditions, such as heatwaves and flooding, increasing amounts of pathogenic bacteria in foods normally followed. Newell et al. [26] highlight that foodborne pathogens, including *Salmonella* spp. and *Escherichia coli*, thrive in novel conditions, for example in fresh produce, and create more challenges to public health like antimicrobial resistance. This further complicates the effects of climate change.

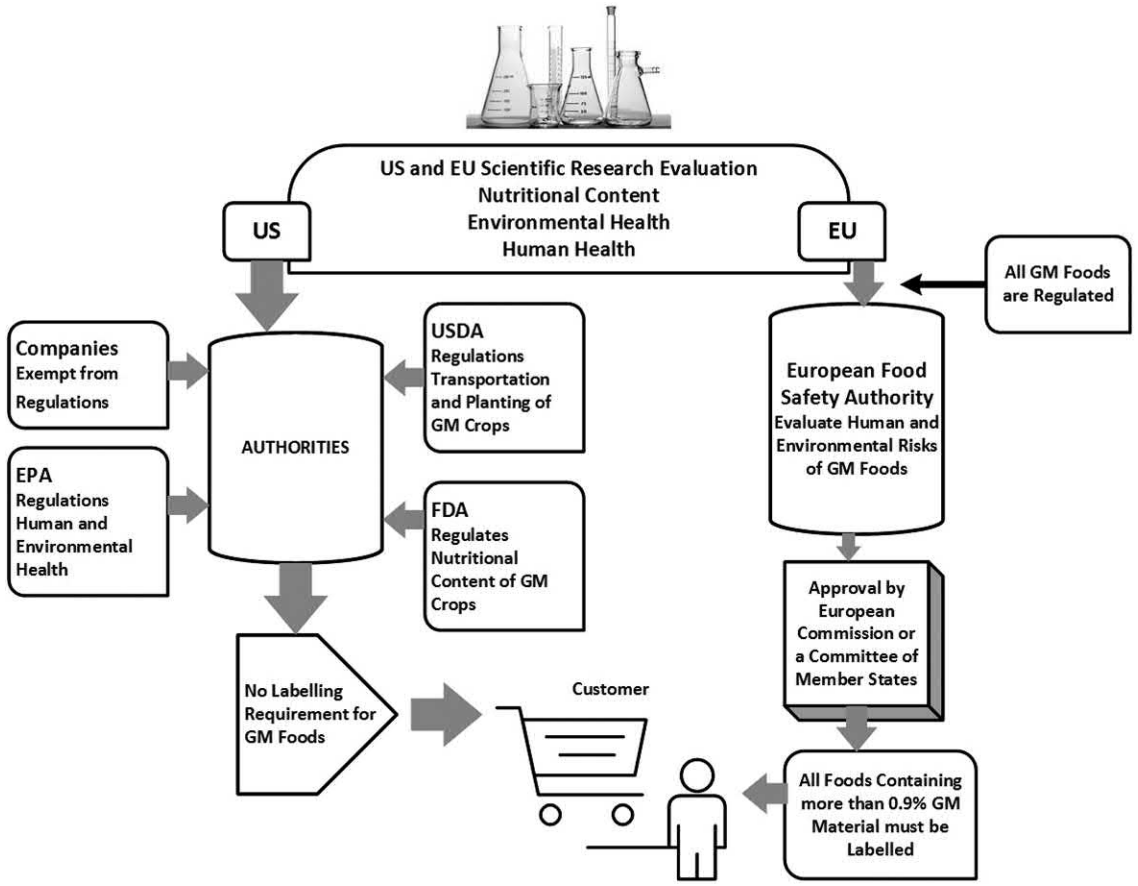

FIGURE 61.1 Differences in food regulation and policies in Europe and America regarding genetically modified foods. (Adopted from Lau [20].)

Jaykus et al. [25] recommend understanding and preparing for the impacts of climate change, as well as constantly monitoring and reviewing the environment, foods, livestock, and wildlife for risks [26], which is important for the early identification and rectification of new issues and trends that climate change and pathogens bring. Early identification will also assist in keeping food safety policies and legislation up to date. Quested et al. [27] relevantly found: "The most important factor in reducing the burden of food-borne disease was identified as our ability to first detect and investigate a food safety issue and then to develop effective control measures."

61.4.3 Technologies

Emerging technologies, such as nanotechnology and biotechnology, serve a number of benefits, inter alia, increasing productivity and the quality of food packaging, production, safety, and taste as well as pest control and disease [6]. However, the current framework will need to be updated as nanotechnology and other technologies remain novel and bear unprecedented risks, such as nanomaterials entering the bloodstream and interacting with organs, causing possible cytotoxic effects [28]. The novelty in emerging technologies like nanotechnology leaves it difficult to define, therefore often difficult to regulate. The European Commission continues to tackle this dilemma as more technologies are adopted [6].

The divergence of international opinion (due to the diverse ethical and cultural values) on biotechnology portrays another difficulty in uniformly regulating such technologies. Article 8(g) of the UN Convention on Biological Diversity (CBD) was introduced to ensure the safety of biotechnologies, particularly where they are a threat to biological diversity and human health. Although the CBD has been ratified by more than 190 countries, only 157 countries have ratified the Cartagena Protocol, Article 19, paragraph 3 of the CBD, which is a legally binding instrument aimed at biosafety [29]. The main reason for this is the effect the protocol may have on a country's trade in GMOs [29]. The protocol has been criticized by some as being ineffective as major players in GMOs, such as the United States and Australia, are not parties to the protocol [29].

McCullum et al. [30] recommend that rather than managing the consequences of new uses of biotechnology after their introduction to the market, dialogue between governments, their citizens, and the private sector should be initiated before research and the development stage. In this manner, resources can be effectively allocated and the wider community can be informed and participate in the progress. Such an approach can also be applicable to the international community.

61.4.4 Conclusion

As food laws and regulations face future challenges, as explored earlier, academics continue to emphasize the importance of monitoring climate change and emerging pathogens. As risks are identified, policies and legislation will need to be updated to reflect these changes, and governing bodies should remain proactive. Food laws will also need to be globally standardized as globalization and international trade increases. In order to avoid conflicts of these international laws and standards with local legislation, international laws should take a legally binding status. International cooperation will also be essential to ensure the enforcement of these food standards globally.

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62 Commercial Considerations: Managing Profit and Quality

Anne Perera and Gerard La Rooy

CONTENTS

62.1 INTRODUCTION

While the handbook is essentially a technical publication written for technical people, it is appropriate to keep in mind the book's fundamental purpose, which can be stated in its simplest form as "caring for the customers." Putting caring for the customers in a practical context, we can say that organizations must have the intent and appropriate capability to develop and produce wanted and risk-free products and services. When we say risk-free, we mean not only risk-free for the customer; however important that requirement is, it is also very much applicable to the organization's objectives. Indeed, the proper management of risk is particularly about not endangering the continuity of existence of the business enterprise. In determining the content of this chapter, we had to make a choice between breadth and depth. We felt it was more appropriate to go for breadth in order to provide the reader with a reasonable range of topics so that anyone interested can undertake further reading as they see fit.

When working on the structure of this chapter, we were mindful of the thrust of this part of the handbook, enhancing food preservation by an indirect approach. For us, this means that food-preservation professionals must be prepared to go beyond their traditional fields of expertise and see their contribution in a much wider content. It also reinforces our view that irrespective of the method of preservation employed, the selection of the method must not only be based on sound technical grounds, but also on appropriate business considerations. Please note that while the chapter as a whole was compiled by us jointly, the specialized sections were contributed by either Anne or Gerard individually.

62.2 MANAGING PROFIT

62.2.1 Business Environment

For a business to be successful, it must have sound processes, up-to-date and profitable products, and well-managed services. Successful companies, besides being highly competent in their respective technical fields, also need to be very skilled in business management. While this need for business skill is generally accepted, it should be appreciated that there has been a fundamental change in what business management ought to be about. Many business structures have changed from "tall" structures to ones with few levels and with fewer "functional silos." This means, for example, that food preservation is becoming more fully integrated with marketing, production, and customer service. To illustrate the points made so far, we will look at the case of "flat-earth thinking" versus "round-earth thinking."

62.2.1.1 Management Structures and Practices

Peter Scholtes of Joiner Associates has likened the traditional style of management with the belief that the earth was flat. He explains that people who believed that the earth was flat would ask questions like: "What happens when I sail my ship until I reach the edge?" Once science proved that the earth was round, a sudden shift in thinking occurred, and questions about falling off the edge became irrelevant [1].

What this means is that organizations that are flat-earth based (and we believe that a considerable number still are) will need to undergo some revolutionary rather than evolutionary change. The revolutionary changes required will make their current management practices irrelevant, redundant, and in many cases quite wrong. However, once the big change in thinking has been accepted and the practices implemented, we can employ the process of evolutionary change for further development and improvement [2, 3].

To illustrate how structures and practices have become irrelevant or inappropriate, we can construct a simple comparative table (Table 62.1). Looking at the table, we can observe that some of the items are either one way or the other, e.g., directive driven or direction led. Other items are more continuous (tall versus flat). If we consider the complete table, however, there can be little doubt that we are dealing with a dichotomous situation—the traditional versus the new.

TABLE 62.1
The Changing Business Environment

Traditional (Flat Earth)	New Age (Round Earth)
Executives near CEO	Executives near staff

Expecting "Traditional" organizations to change gradually into "New Age" ones is a bit like expecting an ocean liner to start sprouting wings and gradually change into a 747 aircraft. Putting it another way, we sometimes need revolution before there can be evolution. These changes in business have had, and continue to have, a profound effect on the food-preservation profession, and practitioners need to be fully aware of these changes lest they be left behind with their flat-earth thinking [4].

62.2.1.2 Changing Role of the Food Professional

The science of food preservation is also undergoing much change. It should be appreciated, however, that we are not just concerned with technological change. On the contrary, we believe that the major changes and challenges facing the profession will continue to come from the changes to the business environment referred to earlier [5].

Consequently, there is a need for the profession to become more and more integrated with the totality of the business. What this means in practice is that professionals have to assume a wider role and accept greater responsibility for the success of the companies that employ them. To identify the specific change in emphasis, which we believe is necessary to ensure the profession's further effectiveness and relevancy, it is useful to employ another comparative table (Table 62.2). While most items in the table are fairly self-explanatory, the question of functional (personal) objectives versus company objectives warrants some examination. For many technical personnel, the main work focus tends to be on objectives that are very close to the person in question and on the quality of the actual process. "Doing things right," it could be called. For example, a product development professional will have expectations about the quality of the development process itself and what is to be ready by when. He or she will also

TABLE 62.2
Conventional and Business-Aligned Approaches

Conventional	Business Aligned
Narrow view	Wider role
Advisory	Accountable
Product emphasis	Customer focus (external *and* internal)
Production driven	Market led
Cost unawareness	Profit appreciation
Quality control	Quality management
Risk avoidance	Quantified assessment of exposure
Functional skills	Business knowledge
Functional/personal objectives	Company/organization objectives

be concerned with the robustness of data and with the appropriateness and quality of any tests and experiments. It is our view that this almost exclusive focus on functional objectives is not sufficient in today's commercial environment, let alone in tomorrow's. What is required of the New Age professional is, in addition to technical competence and focus, a marked increase in appreciation of company objectives, overall results, and the commercial "levers" that drive them. We can term this demand for additional understanding and emphasis ensuring that we "Do the right things." The important point is that unless we do the right things as well as doing things right, our efforts may be ill-directed and as a consequence largely wasted.

62.2.2 Commercial Requirements

Most food-preservation technologists are bound to be employed in a commercial enterprise of one type or another at some time during their working lives. Even for those with entirely academic careers, there is still the issue of continuing demand for greater commercialism in the management of academic institutions. Consequently, it is important for professionals in any technical field to have a reasonable appreciation of business fundamentals as well as the commercial and other expectations of the enterprise for which they work. A proper understanding of these fundamentals and expectations will enable the technologist to contribute to the organization's success to the fullest extent possible [6].

The work of technical staff can profoundly affect, both positively and negatively, the financial performance of most organizations. Sound technical developments can open up new business opportunities, lead to greater efficiency, and secure a stronger position in the marketplace. Misdirected efforts, on the other hand, are likely to result in increased and unnecessary complexity, higher costs, poorer asset utilization, and lower profitability. It is perhaps worth noting that profit is not a dirty word but is in fact a vital prerequisite for growth and long-term success. Profitability is a must if funds are to be available for investment in new products, processes, and technologies.

62.2.2.1 Revenue, Cost, and Assets

Of the many factors that impact on a business's financial performance, the following are the most critical:

Revenue
Costs
Assets employed

Food professionals can and often do have a very significant impact on all three. Before looking at how this may come about, it is important to understand the way the factors affect overall financial performance and their interrelationship.

62.2.2.1.1 Revenue

Assume there are two firms, X and Y, with annual sales revenues of \$50 million and \$40 million, respectively (Figure 62.1). Which is the better-performing company? In terms of sales revenue, the answer is firm X, but more information is needed to determine which is the sounder firm, e.g., costs.

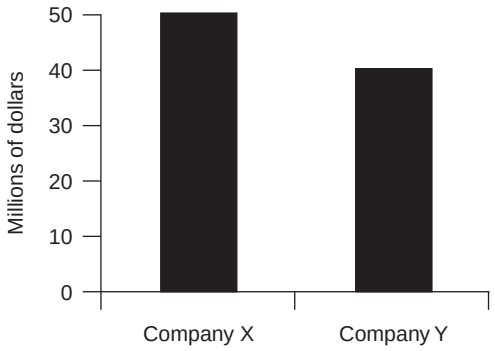

FIGURE 62.1 Revenue of companies X and Y.

62.2.2.1.2 Costs

Assume the costs are \$40 million for company X and \$20 million for company Y (Figure 62.2). With a margin of \$10 million for firm X and \$20 million for firm Y, which is the better company? Looking at just the margin, it is clearly firm Y, but before we award them the annual prize for performance we need to look at the resources employed to generate the \$20 million.

62.2.2.1.3 Assets Employed

When assessing financial performance the resources are considered to comprise the assets employed by the organization, such as buildings, land and plant (fixed assets), and funds tied up in inventories and debtors (current assets—these can be turned into cash reasonably quickly). If the total assets used by the companies are \$20 million for X and \$50 million for Y, which now shows the better performance?

$$\text{Performance} = \frac{\text{Margin}}{\text{Assets}} \times 100\%$$

$$\text{Performance for company } X = \frac{\$10}{\$20} \times 100\% = 50\%$$

$$\text{Performance for company } Y = \frac{\$20}{\$50} \times 100\% = 40\%$$

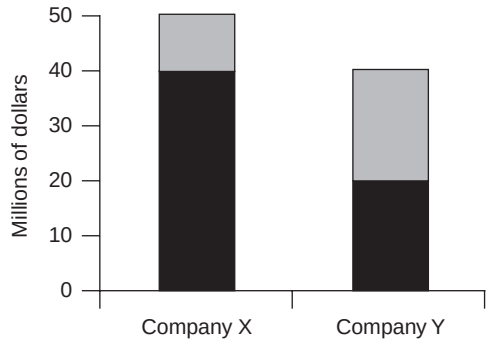

FIGURE 62.2 Costs of companies X and Y.

62.2.2.1.4 Observations

For company X:

The lower margin may mean the business deals in low-value items. Lower use of assets could mean quick turnover of stocks, calculated as follows:

$$\frac{\text{Annual Sales}}{\text{Average Inventory Value}}$$

If, for example, half of the $20 million were tied up in inventories, the stock turnover per year would be 50/10 = 5 times per annum. The low asset figure may also mean low plant book values (plant could be obsolete). The old plant may be costly to operate and hence be the reason for the low margin.

For Company Y:

This company appears to deal in higher-value items. The higher margin may attract competitors to move in on the market. Stock turnover (again assuming half of the assets are tied up in inventory) would be 40/25 = 1.6 times per annum. The company may have a more modern plant, which may mean a lower cost structure and hence the higher margin.

62.2.2.2 Fixed and Variable Costs

Many actions and decisions made by technical staff have a significant effect on costs. It is therefore important for the decision makers to appreciate the difference between fixed and variable costs. Let us consider the basic definitions first.

62.2.2.2.1 Fixed Costs

In brief, fixed costs are those costs not affected by changes in output. An example of a fixed cost would be the cost of monthly machine rental, which we would incur no matter what the output. Note, however, that any production beyond machine capacity would mean a step in our fixed costs, i.e., cost of renting another machine. Also, the passage of time can lead to increases or decreases in fixed costs. Consequently, the earlier statement on fixed costs needs some qualification: Fixed costs are not affected by changes in output within a specified output range, and fixed costs remain constant within a specified period.

62.2.2.2.2 Variable Costs

Variable costs vary with changes in output, and the unit cost is generally deemed to be constant, although that is by no means always the case in reality. For example, bulk purchase may bring the cost per unit down, or in other cases the cost of additional units may be higher than the average. When comparatively small quantity ranges are considered, however, variable costs may be taken as truly variable, i.e., the cost per unit stays constant and total variable cost can be found by multiplying the unit costs by the quantity used.

62.2.2.2.3 Fixed versus Variable Costs

In many business situations, including the technical field, there are often trade-offs to be considered between fixed and variable costs. It is important to have a clear understanding of the difference between fixed and variable costs as well as the relationship between the two. Assume a company wishes to launch a new product and has been given two different manufacturing proposals.

1. Proposal 1: Low fixed cost, high variable cost
   Fixed cost (equipment, etc.) = $100,000 pa
   Variable (material, labor) = $10 per unit
   Selling price = $15 per unit
   Sales volume = 40,000 units pa
2. Proposal 2: Higher fixed cost, lower variable costs: The second proposal is more mechanized and hence requires less expensive material and less labor. Fixed costs are consequently $100,000 higher, but variable costs are lower at $7 per unit.
3. Initial comparison: The two proposals can be readily compared by means of a table (Table 62.3).
4. Comparing profit and break-even points: To determine which of the two proposals is better in terms of the data supplied, we need to calculate (i) total profit for the year and (ii) the break-even point, i.e., the volume at which total revenue equals total cost. Again the two proposals can be compared (Table 62.4).

TABLE 62.3
Comparing Fixed and Variable Costs

	Proposal 1	Proposal 2
Fixed cost pa	$100,000	$200,000
Variable cost per unit	$10	$7
Selling price per unit	$15	$15
Sales volume units pa	40,000	40,000

TABLE 62.4
Comparing Profits and Break-Even Points

	Proposal 1	Proposal 2
Sales revenue = quantity × selling price	600,000	600,000
Fixed cost	(100,000)	(200,000)
Variable cost = quantity × variable cost per unit	(400,000)	(280,000)
Profit	100,000	120,000
Contribution margin = selling price – variable cost	$5	$8
Break-even point = fixed cost ÷ contribution margin	20,000 units	25,000 units

5. Observations: While proposal 2 is more profitable, proposal 1 is less risky. If sales are evenly spread throughout the year, it would take the first proposal 6 months to break even, while for proposal number 2 the break-even point would occur at 7½ months. The break-even analysis, while not a sophisticated tool, is quite useful in illustrating the differences between proposals. Take care, however, as in reality, fixed costs have a habit of going up (or sometimes down), for example, when

needing to rent an extra piece of equipment. Also, variable costs are rarely as linear as assumed here, although as said earlier, they can usually be taken as linear between reasonably small quantity intervals.

62.2.2.3 Price, Margins, and Costs

Before discussing the impact of technical staff on costs, it is necessary to introduce a number of cost-related concepts. The approach to the management of costs in an organization is very much dependent on how costs are viewed—as a starting point or as the endpoint. Figure 62.3 illustrates different ways of considering costs.

62.2.2.3.1 Traditional Cost Plus

The traditional method of determining a selling price was to add up all direct and indirect costs (e.g., overheads) and add a suitable margin. This approach works when there is little or no competition (e.g., due to import control). Unfortunately, some companies will persist with this approach even when, because of changed conditions, it is no longer appropriate (Figure 62.3, method 1).

62.2.2.3.2 Market Price

In a free market, the price is set by that market and has nothing to do with costs or margins. The change from "tradition" to "market price" method took place in some countries during the 1950s and 1960s but in many other countries during the 1970s and early 1980s. The principal effect of the change to a market (and as a rule lower) price was reduced margins, since costs were generally considered as "fixed" (Figure 62.3, method 2).

62.2.2.3.3 Required Margins

The squeeze on margins meant that many organizations experienced unsatisfactory returns on their invested capital. However, to attract new capital, returns and hence margins must be adequate. Since prices are largely beyond the control of the organization and margins must be achieved, the real situation is that:

$$\text{PRICE} - \text{MARGIN} \rightarrow \text{COST}$$

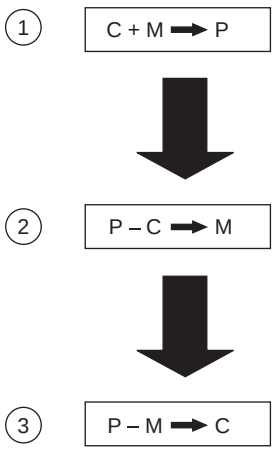

FIGURE 62.3 C, M, P relationships, where C = cost, M = margin, and P = price.

This means that cost is the most important element and one that management needs to focus on constantly.

62.2.3 Technical Impact on Business

Most managers do not fully appreciate the impact that technical resources have (or could have) on the business in general and on revenue generation in particular. What is more, it is quite likely that the majority of technical staff are also not completely aware of their contribution to the success of the enterprise. We will look briefly at four types of impact:

Impact on revenue
Impact on costs
Impact on assets
Impact on cash

62.2.3.1 Impact on Revenue

In many cases, the technical staff provide the lifeblood for the organization's future, as all products and processes require constant renewal and replacement if there is to be security of future revenue.

62.2.3.1.1 Types of Impact

When considering the impact that technical professions have on an organization's revenue generation, it is necessary to appreciate that there are shorter-term and longer-term impacts: (i) Shorter-term impacts (tactical or current revenue): Projects coming under this heading are generally concerned with existing products and processes or new products within existing competencies (e.g., product line extensions, product improvements, ingredient substitutions, etc.). (ii) Longer-term impacts (strategic or future revenue): These are concerned with new technology and products outside current competencies.

62.2.3.1.2 Conceptual and Analytical Soundness

An important point to note is that a project can be analytically correct but conceptually wrong at the same time. In other words, the arithmetic is fine but the underlying premises are flawed. Technical staff should seek advice if required and take particular care in (i) setting out all underlying assumptions clearly, (ii) defining the scope of the project accurately (including in some cases any matters which, while outside the project, may still be relevant), (iii) listing all agreed expectations in terms of both magnitude and probability, and (iv) assessing the economies of the proposal soundly and presenting the justification in a logical manner.

62.2.3.1.3 Reliable Tangible Cost and Benefit Information

Proposals need details about the various types of costs and benefits, that is: One-off costs and benefits (e.g., purchase of new packaging printing dies, the sale of some piece of equipment made possible by the project) and ongoing costs and benefits (e.g., increased production energy costs, reduced labor costs).

62.2.3.1.4 Intangible Cost and Benefit Information

It is important to include all intangibles, even if it is difficult to estimate dollar figures. It may in fact be better not to try to show dollar figures, as it often leads to much argument and debate. Make sure, however, that all intangibles are clearly identified and list them separately, preferably at the end of the presentation. Examples of intangibles include:

Enhanced brand image
Improved competitive position
Increased customer satisfaction
Improved staff morale
Adverse impact on the quality of life of people in the neighborhood
Waste generated by proposed process not suitable for recycling

The point to remember is that intangibles should not be dismissed as of little importance because of our inability to put precise dollar figures on them. On the contrary, with many projects the intangibles (the "soft" issues) are often more important than the "hard" (dollar) issues.

62.2.3.1.5 Technical Revenue Contribution Reporting

When it comes to ongoing management, technical function professionals may assist their cause by ensuring that the revenue contribution is duly reported on and acknowledged (Table 62.5).

62.2.3.2 Impact on Costs

Technical staff can and often do have a very significant impact on costs. Frequently the technical professionals are the members of an organization who make the decisions that affect not only the total costs of a development but which more particularly determine the balance between fixed and variable costs. Understanding that balance is important especially when dealing with new proposals. Some examples are outlined below.

1. Increased R&D costs: Deciding to spend more on R&D (higher fixed cost) in order to gain lower product costs (variable costs).
2. Higher specification levels: Insisting on a higher specification than may be necessary is likely to push up the costs of production (higher variable costs). The decision can also result in higher fixed cost if, for example, the close tolerances specified require a more expensive piece of equipment than would be the case with a less demanding specification.
3. Product line extension: Every time a new product is added to a company's product portfolio, it is likely that production run sizes and frequencies will be affected (usually adversely). Consequently, the set-up cost (a fixed cost) has to be recovered over a smaller number of units, thus driving up the total cost per unit.

62.2.3.3 Impact on Assets

We have already considered briefly the importance of the assets employed when assessing a company's performance. As with costs, the actions and decisions of the technical professional can have considerable impact. Again some examples can illustrate the position.

62.2.3.3.1 Current Assets

When launching a new product, a company invariably needs to increase its working capital (current assets), for example, (i) extra funds tied up not only in finished goods but also in new ingredients and components, and (ii) additional funds required to finance the increased debtors resulting from the sales of the new product.

62.2.3.3.2 Fixed Assets

Technical developments often lead to companies investing in new processes and equipment. It is important that this new investment does not dilute the company's financial performance, that is, the return on the new investment should not be less than the norm for that particular industry and preferably be better than the company's current average return. Exceptions may occur when companies invest in new processes and facilities for strategic reasons, provided of course that this fact is understood and accepted by the decision makers.

62.2.3.4 Impact on Cash

Companies may be profitable but still fail if they run out of cash and cannot pay their bills. While a proper discussion on cash management is beyond the scope of this chapter, it should be noted that profit and cash are not the same things. It is important that the technical professional has some understanding of that difference, since again technical decisions

TABLE 62.5
Sample of Technical Revenue Contribution Report

	Year to Date	Budget	Variance	Last Year	Estimate for Remainder of Year
Net sales $ "old" revenue					
Net sales $ "new" revenue					
Total net sales					
% New revenue of total net sales					
Technical costs					
% Technical costs of new revenue					

can have considerable impact. (If you wish to pursue this topic, we suggest you talk to the financial staff in your organization about how organizations absorb and release cash. You may also like to find out about cash flow evaluations).

62.2.4 Technical Responsibilities

Companies employ food professionals to carry out specific roles in various technical areas, such as research and development, quality assurance, and technical services.

In these roles, food professionals have the opportunity to put into practice what they have learned during academic training. Professional development, recognition, and experience gained are part and parcel of the rewards.

62.2.4.1 Research and Development

Research and development, commonly referred to as R&D, is a vital activity for any progressive company. Failure by companies to develop new products and processes will not only hamper growth, it may lead to the decline and possible demise of the organization. We believe that this is especially so in the case of food companies.

Food companies depend on their technically qualified professionals to carry out the functions of new product/process developments to move their companies forward. This is achieved by identifying products/processes that have the potential to increase the profitability of the company. The research and development function of a food company must thus be closely associated with both marketing and production functions. New product ideas are generated by companies through

Study of market trends
Brainstorming
Requests from customers
Competitive pressures

Ideas that show promise and fit the company's criteria are passed on to the R&D department for development.

62.2.4.1.1 Product Development Processes and Procedures

The product development process will depend on the type of company, the nature of the business, the company philosophy, and management style. Some companies have a formal procedure in place to ensure that product/process development is carried out systematically, while others may develop new products in an informal manner.

As a rule, a more formal approach gives better and more reliable results, especially in larger companies. The main steps comprising a product development procedure are shown in Figure 62.4. Since developing new products is generally an expensive process, companies must subject all new ideas to rigorous screening and prioritizing to ensure that only those ideas with a high probability of success are approved for development. Also, if a partially developed product is no longer likely to meet expectations it is better to abandon the idea. The costs incurred must be considered as "sunk costs," and it would be incorrect to spend any more resources. Another point to note is that products developed at considerable expense principally as technical projects but without a proper input of marketing requirements or with insufficient understanding of business objectives, are unlikely to see the light of day. It is possible to prevent such situations by a formal approach in managing the product development process.

62.2.4.1.2 Product Development Considerations

Food professionals as members of an R&D team need to have access to information on new product launches in their specific product categories, preferably from around the world, and set up a product-awareness library. They also need to build their own networks with other professionals in the areas of ingredients, packaging, and analytical laboratories related to product quality in terms of food composition, and nutrition. Some large food companies have their own technical centers and in-house support services, while others have to depend on outside facilities to provide such services. In dealing with organizations and personnel outside their own company, food professionals involved especially in new product development need to be aware of the importance of maintaining confidentiality about commercially sensitive information.

Professionalism in R&D requires practitioners to be familiar with rules and regulations pertinent to their area of work and to abide by them at all times. There are no universal food regulations, and it is the responsibility of the food professionals to know the food regulations of their own country as well as the relevant information on regulations of countries importing their products. In this regard, it is important to understand that different countries have different labeling requirements. While the artwork on packages is normally handled by the marketing department, R&D personnel have to take an active role in providing and/or approving information that is to be shown on the labels of new products.

In addition to new product development, most food companies carry out continuous product improvement through ingredient substitution and/or process improvement. No matter which R&D activity food professionals are involved in, they need to be aware of the cost implications and try to avoid unnecessary expense. Developing product specifications is a function that R&D staff must carry out with essential input from the marketing and production departments. Such teamwork is necessary to ensure that the marketing expectations are matched by the production capabilities of the company. Once the specifications are developed and approved, they become the basis for quality assurance, which is another area in which food professionals are employed.

62.2.4.2 Quality Assurance

The primary role of the quality assurance (QA) function is to assure that the quality of the products processed and marketed by the company is of the standard required, in other words, that the products are made in accordance with specifications. It is the responsibility of the QA function to ensure that the raw materials received comply with the standards that have been mutually agreed to by the company and the supplier. If

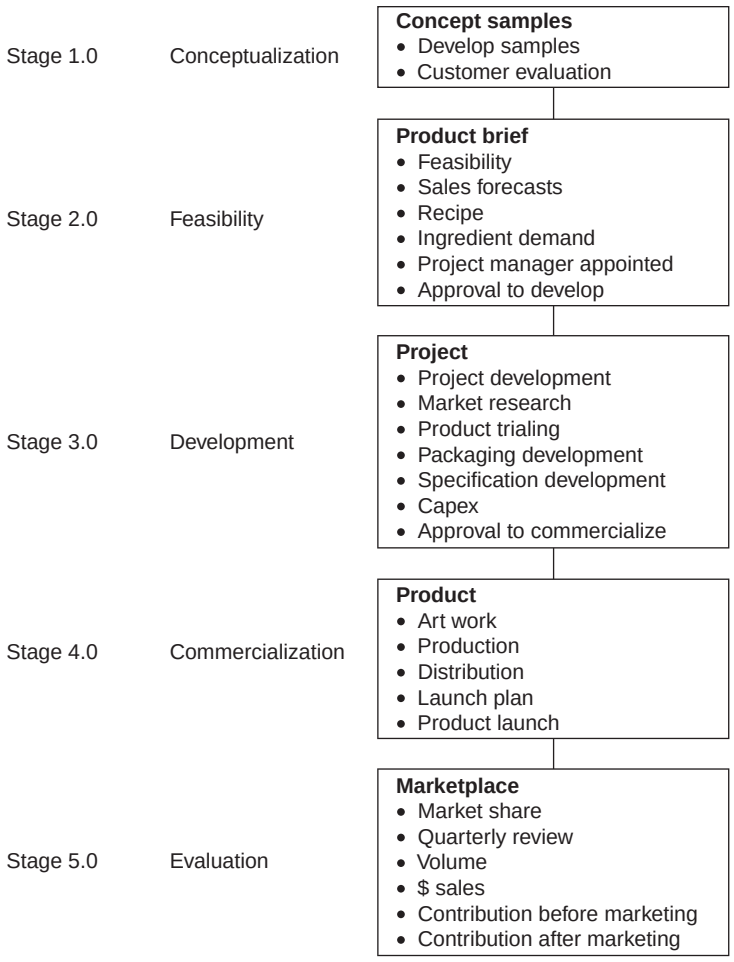

FIGURE 62.4 Main steps in product development procedure.

the incoming material is not of the agreed quality, the QA department should intervene and take the necessary action. In most cases, this means rejection of the incoming material. Only in exceptional circumstances should reworking be considered. In any case, reworking is, as a rule, a costly exercise, which should be avoided if possible.

During processing, samples are generally taken for routine checks of quality. Specific quality parameters of the product in question are recorded on QA forms, which are held in a file. This enables the product history to be traced back if required. Such records also provide valuable process performance information and may serve as evidence when responding to consumer complaints and/or in the event of a product recall. Staff involved in QA must take all precautions and all necessary steps to prevent any problems from occurring. Such problems may be related to the contamination of a product with undesirable substances, be they of physical, chemical, or microbiological origin. Programs such as Hazard Analysis Critical Control Point (HACCP; see Chapter 57 have become increasingly important to food companies in monitoring and preventing potential problems. It should be noted that hazards are not confined to just the actual food products. On the contrary, manufacturing hazards can have significant impact on the environment.

62.2.4.3 Other Technical Services

Research and development with quality assurance are not the only technical functions of food professionals. For example, those with interests in sales and marketing can become technical sales personnel or product managers. Their skills are valuable in providing technical background on the products, ingredients, and equipment/machinery that they sell or market. In addition, they are in a good position to relate to a client company's R&D and QA staff, who generally are the customers for such goods and services.

Although cost is often a considerable factor in decision making, in many instances the decision of a client company to purchase from one supplier and not the other is determined by the type of technical service provided. Leading ingredient companies employ food professionals to work closely with their clients and often produce tailor-made ingredients, products, or equipment so as to suit their specific needs. Some food professionals from supplier companies work in close association with the product development staff of client companies in developing new products. In such cases, the client company may negotiate exclusive rights for the ingredient and or the production system for a given period of time. In situations where new ingredients are not yet permitted by the regulatory

authorities of some countries, the supplier companies or their agents in the countries concerned may intervene and often succeed in obtaining approval. Food professionals play an important role in preparing submissions for approval, which often require substantial technical input by way of research and supporting evidence on the safety of the ingredient in question.

62.3 MANAGING QUALITY

62.3.1 Quality Scene

Over the last two decades, there has been a considerable increase in the understanding and acceptance of the importance of quality. The technical professional needs not only to be in tune with this change in thinking, he or she must also take a proactive stance because it often is the technical area in which one finds the origins of both quality successes and failures as well as the remedies.

62.3.1.1 Importance of Quality

To assess the nature (or the customer's perception) of quality, it is useful to employ Dr. Kano's model of quality, which identifies three different aspects of quality: Basics, satisfiers, and delighters.

62.3.1.1.1 The Basics

The basics are the fundamental aspects and features of a product (or service) that are considered to form part of its inherent functionality. Basics are all those ordinary things that the customer expects to be up to the mark (e.g., size, flavor, functionality). The important point to realize is that failure to meet the basics usually makes the customer angry.

62.3.1.1.2 The Satisfiers

Satisfiers are those aspects and features that make the customer feel happy about his or her purchase. They are important to the customer but are only effective if the basic requirements have been met.

62.3.1.1.3 The Delighters

Delighters are those extra features or services that clearly exceed the customer's expectations. They can, however, be counterproductive if the basics are not met. A faulty product coupled with a delighter will make the customer cynical.

62.3.1.1.4 Points to Note

The following are worth noting: (i) most customer complaints are likely to be concerned with the basics, (ii) remember, failure to meet the basics tends to make the customer angry, and (iii) the quality model is very relevant in all areas of management, be it marketing, product development, or production.

62.3.1.2 Origins, Sources, and Causes of Quality Problems

Quality problems usually manifest themselves when a customer complains or when quality assurance staff detect an out-of-specification product. To find the root causes of complaints we need to dig deep. It is a bit like peeling an onion—each layer needs to be removed before the next one is revealed.

62.3.1.2.1 Origins

First, we need to identify the three fundamental origins of quality failures. These are (i) faulty design (design mistakes, specification shortcomings), (ii) faulty execution (faults in manufacture or assembly, shortcomings in delivery or service), and (iii) incorrect use (intentional or unintentional).

It should be noted that many, if not most, of the quality failures experienced by firms are the result of faulty design, even though they appear to have their origins in faulty execution. For example: (i) in many cases firms settle on design features and specifications without having a proper understanding of what the operational systems are capable of delivering in a consistent manner, e.g., unrealistic mix tolerances, (ii) quite often designs will carry within them the seeds of faulty execution: Parts that can be assembled incorrectly, awkward design that will encourage assembly shortcuts, unwarranted production complexity, and lack of standardization.

62.3.1.2.2 Sources

Once we have identified the fundamental origin of failure, we can look for the source by identifying each specific process, operation, or procedure involved with the failure. In most instances, we will need a concise description of the process or operation concerned. When faulty execution is the origin of failure, two types of sources are possible: Those within the organization and hence under our direct control (internal), and those outside of the organization, usually because of faulty materials or services (external). Even where the source is external, there will always be some contributing internal factor. For example: (i) When a supplier is responsible for poor-quality components (external source), we also need to identify the appropriate internal contributing factors (e.g., inadequate or unrealistic purchasing specification, price-only purchasing policy, or undue emphasis on credit). (ii) Management of the inward goods acceptance process is inadequate. (iii) The line staff is unable to react quickly when faulty materials are encountered.

62.3.1.2.3 Causes

While having an understanding of the origins and sources of quality failures is a necessary first step, there is little we can do about prevention and improvement unless we also understand the cause of our problems. All quality problems arise from a failure to meet customer expectations. Most of the time a failure is the result of variability in one or more processes. While the question of process variability is discussed in more depth in Section 62.3, it is worth noting here that understanding the variability of processes is an essential prerequisite to reducing quality problems. To reduce the variability in processes we need to appreciate what causes it. There are two different types of causes of variability: Common causes and special causes.

Common-cause (statistical) variability includes the random variation in results or performance, which is due to the

system itself rather than any specific action. This variation is considered "normal" for the particular system. As common-cause variability is an inherent feature of the process, improvement in process performance is dependent on making changes to the system. Pep talks to staff, exhortations, or slogans are quite useless in this situation. Unless the actual system is changed for the better, variability will not decrease. Special causes are those extraordinary and often one-time events that cause a temporary increase (or decrease) in variability. There is no need to change the system of operation to avoid variability due to a special cause.

62.3.1.3 Quality Culture and Processes

It is important to note that in the end, improved quality can only come about if there exists in the organization an all-pervading quality culture. This means not quality one day and quantity the next. It means that the entire organization should be quality aligned with regard to management practices and systems. In addition, there needs to be an organization-wide agreement to regard quality issues principally as opportunities for improvement rather than as problems to blame on someone. In practice, it means that all quality issues need to be viewed in terms of origin, source, and cause (as outlined earlier) and that there are in place sound, standard, and accepted process-improvement techniques in the company. It is important to appreciate that it is only when the underlying processes are improved in a permanent fashion that one can expect a sustainable increase in quality performance.

62.3.2 Understanding and Reducing Variability

It is very important for technical staff to thoroughly appreciate the effect process variability can have on business performance. There are two principal impact areas:

1. At the time of product and process development, the R&D specialist has a major role in ensuring that potential process variability is understood and minimized.
2. During production, the quality assurance professional can do much in introducing the right monitoring systems and training so that operational staff can learn about process performance and subsequently work to reduce its variability.

62.3.2.1 Costs Associated with Variability

To introduce how variability can reduce quality and performance, and drive up costs, we will use as an example the costs involved in maintaining a painted house. The lasting quality of paint on a wooden house provides a classic demonstration of the effects of variability in performance and the costs associated with it.

62.3.2.1.1 Variability in Paint Performance

Have you ever had the unenviable task of preparing a clapboard house for repainting? You must have wondered as you toiled away why some of the paint had flaked away while other bits stubbornly remained in spite of vigorous wire brushing and scraping. You may also have seen houses that obviously haven't been repainted for several decades yet still have some paint showing. While some of the remaining paint would be easy to peel off, other remnants would still be difficult to remove.

62.3.2.1.2 Costs of Paint System Performance

Consider the case of a house with painting costs over the first 35 years of its life as shown in Table 62.6. If there was no variability in the performance of the paint, all the paint would last exactly the same length of time. Remember that some small parts of the paint system remain intact for several decades, so if all the paint performed as well as the best parts, a 35-year life could be possible. If there was absolutely no variability, we could predict the life of the paint system with total confidence. We could plan the job of painting (Job A) with precision, as on a given day (known in advance) all of the paint would fall off the house. In addition, there would be no need for scraping and sanding. The table shows that the difference in per m^2 cost between the two scenarios is \$32 (\$48 – \$16), which is the cost due to the variability in performance of the paint system. Even if we take a more modest paint life of 2l years, the cost would still be considerably less. What the example shows very clearly is that there are costs associated with variability and that high variability means high costs.

62.3.2.1.3 Other Costs

While the dollar costs associated with high variabilities are very important, they are in the majority of cases reasonably self-evident and quantifiable. But there can be other important costs.

1. The costs of a poor reputation: Without any doubt the most important of all costs is the detrimental effect variability has on one's reputation in the marketplace.

TABLE 62.6
Costs Associated with Variability in Paint Performance

			$ per m^2 (labor and material)
A. When new: Three-coat finish			8
B. After 7 years: Making good spot failures, spot prime, and two coats			7
C. After 14 years: Making good spot failures, spot prime, and two coats			12
D. After 21 years: Complete repaint including sand back, refill prime, coat			12
Paint life costs:			
Year	Job	$	Accumulated
0	A	8	8
7	B	7	15
14	C	7	22
21	D	12	34
28	B	7	41
35	C	7	48

If there is one thing that annoys customers more than anything else, it is a lack of consistency. Nothing will damage a reputation more quickly and more permanently than a high level of variability in the quality of products and services.

2. Staff-related costs: These costs are associated with pride of workmanship and staff morale. High variability in our processes is likely to cause many internal problems, such as scheduling difficulties, quality-of-fit problems, and reworking—all of which are bound to lead to reduced job satisfaction and, as a consequence, to poorer performance.

62.3.2.2 Managing and Reducing Variability

In many organizations, process variability is given insufficient weight, resulting in quality problems and subsequent loss of customer confidence. It should also be appreciated that variability reduction is not something that happens by itself. On the contrary, it requires focused management attention, sound systems, and properly trained staff.

62.3.2.2.1 Reasons for Variability in Performance

To understand why we often experience quite a large variability in performance, we need to identify the various sources. In the case of the painted house, they can be divided into four categories:

Timber surface: The original timber surface is likely to be a source of considerable variability in the performance of the paint system. Variation in surface finish, moisture, and resin content will all contribute to that variability.

Materials: If you buy paint supplies from a reputable source, you can be fairly certain that the variability will be low. However, in spite of a manufacturer's best efforts to keep paints uniform, there will always be some variability. This is likely to be greater if a particular paint comes from more than one batch. The upshot is that some of the paint will perform better than average and some worse.

Application: The way the paint is applied provides an enormous potential for variability in performance. Using a brush, for example, results in large differences in coat thickness. Even employing different painters for different parts of the job is likely to lead to an increase in variability. In addition, one must take into account the varying painting conditions, such as air temperature and humidity, which will increase variability in performance.

Position: This is a critical factor in how long a paint system is going to last. Areas of paint exposed to wind, rain, and full sun are likely to fail earlier than those protected by porches or eaves.

62.3.2.2.2 Understanding Variability

When advocating the reduction of variability, we should remember that most if not all regular company reporting is about reporting averages. For example, most standard costing systems are designed to report average monthly performance against some predetermined (average) standard. Averages by their very natures will mask variability, so while we may aim to meet a particular better average performance, we could be unwittingly steering our ship in the wrong direction.

Take, for instance, a case involving three teams with the average daily outputs over a month as shown in Tables 62.7 and 62.8: On the face of it, Team B is the best, and if we did not look further we could conclude that Teams A and C should pull their socks up and work like Team B. If we were to dig a little deeper, we would learn that the highest and lowest daily figures were as shown in Table 62.8. If we also learned that production in excess of 1200 units per day was considered to be too hard on the equipment and that such high rates of throughput tended to lead to quality problems, which is now the better performing team? In terms of output variability, it is Team A, because it turns in the most consistent performance. Perhaps Teams B and C could learn something from Team A about how to avoid very low throughput days, while Team A could be encouraged to talk to Team C about what they do to reach 1200. As for Team B, the first aim needs to be "no production beyond 1200 units per day" with the subsequent focus on reducing throughput variability further by improving low-day output.

62.3.2.2.3 Best, Worst, or Average Performance

In the case of the painted house, it is quite obvious that the worst-performing parts of the paint system determine the painting costs over the lifetime of the house. The best-performing parts are those difficult-to-remove bits that we strike when trying to get the house back to bare wood. While the average performance of the total paint system could be 20 years, is this useful information? After all, would a paint system with an average life of say 25 years necessarily be a better proposition? What about if in the latter case the performance range was 5–45 years (rather than 7–33 years)? Averages can be highly dangerous statistics, and while politicians and other public figures can be masters at using and misusing averages, in business we see much misunderstanding as well.

62.3.2.2.4 Accumulation of Effects

Because customers experience the final result of the various processes, one needs to be aware of the potential accumulation

TABLE 62.7
Case Study of Three Teams: Average Output

	Team A	Team B	Team C
Average (units/day)	1000	1100	1000

TABLE 62.8
Case Study of Three Teams: Highest and Lowest Output

	Team A	Team B	Team C
Highest daily	1100	1500	1200
Lowest daily	900	700	800

of the variability effects of all processes. Considering the case of the painted house again, the accumulation of effects can be looked upon as a lottery. Some areas are unlucky, in that they have a combination of all of the worst aspects, while others have a combination of the best with the best. Between the two extremes, we have a multitude of combinations, which provide either better or worse-than-average performance. As a consequence, we see some early failures occurring in quite unexpected places (e.g., away from the weather), while other areas of paint continue to perform well in spite of being much more exposed.

If we apply this concept of accumulation of effects to several business processes, we can see why some customers can be "unlucky" when it comes to customer service. Take, for instance, the following situation. We supply goods to customers and have the following performance statistics: Stock availability 90%, order accuracy 90%, invoicing accuracy 90%. Table 62.9 shows the average effect on our customers (assuming the three statistics are independent of each other). We can observe that we have on average one "unlucky" customer per 1000 orders who experiences the "worst with worst with worst" combination. They are the ones who are likely to tell their friends "Don't deal with that firm, they cannot do anything right." Using this example, it is clear why some people end up with a "lemon" of a car, while other buyers of the same model are very satisfied. In the business of food preservation, the problems are very much the same.

62.3.2.2.5 Improvement Opportunities

The important point to note is that process variability will, generally speaking, not reduce by itself. On the contrary, it requires special effort and attention to detail. There are many techniques available, but any detailed discussion is beyond the scope of this chapter. We will therefore confine ourselves to identifying opportunities to reduce variability and to the principles underlying improved process performance.

62.3.2.2.6 Minimizing Potential Variability

It is by seeking to minimize variability in the first place that the technical professional can make a significant contribution to the ultimate performance of the product. During the development phase, there are many opportunities for this, but unfortunately the opportunities are seldom taken advantage of to their full extent. Some examples include:

TABLE 62.9
Customer Performance Statistics

Problems	Percentage
None	72.9
One	24.3
Two	2.7
Three	0.1
Total	100

Understanding the capability of the processes and developing the product and specifications accordingly. There is little point in specifying a process to ±1°C when the actual variability is ±2°C. Looking for opportunities to standardize materials and processes wherever appropriate. Undue and unnecessary diversity does invariably lead to higher variability of output. Making the definition of the process control methods and information a required part of the product or process design and specification.

62.3.2.2.7 Minimizing Operational Variability

Assuming we have taken all important steps to minimize potential variability, we must now put in place the systems and procedures necessary to understand and minimize operational variability. The main point is that processes must be monitored and properly understood before changes and adjustments are made, as shown in Figure 62.5.

62.3.2.2.8 Benefits of Reducing Variability

In spite of what intuition may sometimes tell you, reducing variability through systematic improvement of the process will invariably lead to reductions in cost, increased customer satisfaction, and other benefits.

Lower costs: The ability to set a lower target weight when packing a valuable product and being sure that that customer is not shortchanged.

Happier customers: Lack of consistency in products and services will harm a company's reputation. Customers will be happy when their expectations are understood and satisfied. If you aim to exceed their expectations, be confident that you can exceed them in a consistent manner. Make sure the statement "exceeding customer expectations" is not just a slogan. Raising customers' expectations without the possibility of consistent delivery is bound to have an adverse effect on your business.

Inventory savings: Reduced product variability also makes possible savings in inventory. Improved consistency is a prerequisite for just-in-time (JIT) management. It should be remembered that "out of spec" products as a rule become a liability to the company, requiring "write-downs" or additional expense or both.

Increased capacity: Processes that are under proper control as a rule operate better with resulting increases in output.

Happier staff: Reduced process variability means fewer production problems, less panic, and fewer complaints, all of which help staff to feel better about their jobs and themselves.

62.3.3 Managing Customer Complaints

It is no secret that many companies do not like receiving customer complaints. They are usually seen as embarrassing, troublesome, and possibly frivolous. But we need to manage them well if we are to maintain good customer relations and turn the complaints into opportunities for improvement. To do this we need to (i) ensure we have the correct culture, (ii) design the right system and operate it properly, and (iii) carry out proper analysis, report, and follow-up.

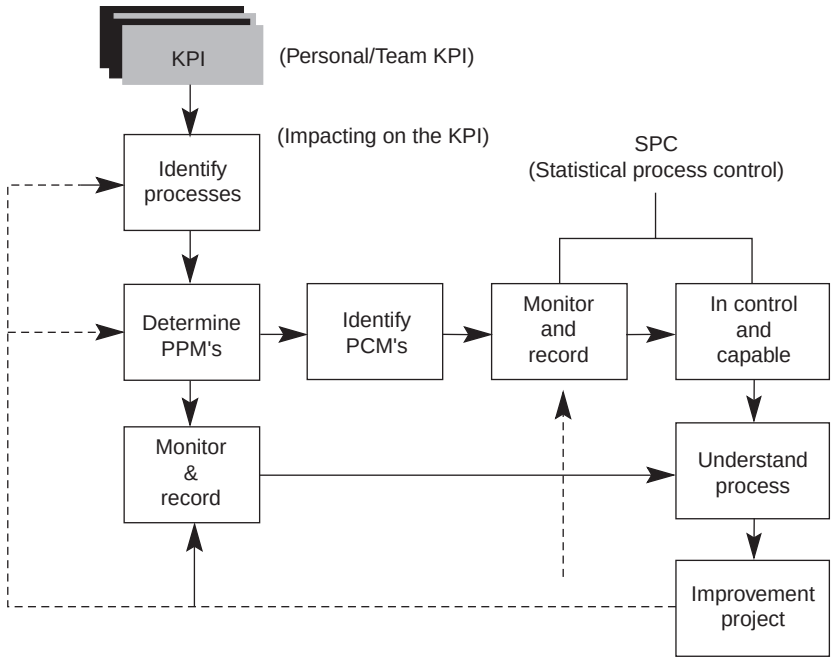

FIGURE 62.5 Systematically reducing variability. KPI = key performance indicator, PPM = process performance measure, and PCM = process control measure.

62.3.3.1 Assessing the Situation

62.3.3.1.1 Correct Culture

Before one designs and implements any system, it cannot be overemphasized how important it is to have the correct culture in place. This means treating a customer who complains as a person doing us a considerable favor. The customer is telling us something about our product or service that we probably did not know but ought to. We should accept that many other customers may have similar feelings about our product or service except that they do not bother to complain. Instead, they decide not to buy the product again, as well as telling their friends about their unhappy experience.

Points to remember include:

- Are customer complaints principally seen as a nuisance?
- Who is responsible for managing complaints, and who actually handles them?
- Are complaints dealt with by some junior and possibly untrained person?
- Is it possible for customers to get "shoved around"?
- Are we sure that complaints are handled professionally every time?
- Are complaints seen as important?

A good check on the perceived importance of customer complaints is to look at how they are reported. Are they included in the monthly report? If yes, are they hidden somewhere at the back, or do they form part of the important summarized results?

62.3.3.1.2 Do We Have the Right System?

Once an organization has accepted that customer complaints are extremely important and introduced the necessary managerial and cultural changes, it should ensure that it also implements a sound customer complaint-management system and related procedures. The most important requirement is confidence that the system and procedures will ensure the appropriate response in every situation. In addition, the procedures should ensure a good standard of recording to provide the organization with a proper source of information for any required follow-up and subsequent improvement of the processes. If they are not up to scratch, redesign them.

Points to consider include:

- Is there in place an up-to-date written procedure for dealing with complaints?
- Does the procedure ensure an appropriate response in every case?
- Can we be confident that all (or at least most) of our customers are happy with the way the organization deals with complaints?
- Does the system ensure proper recording, monitoring, and reporting?
- Do the complaints lead to improvements?

62.3.3.2 Developing a New Approach

If an organization considers customer complaints to be important, it must be prepared to develop, implement, and maintain a sound management system.

62.3.3.2.1 Defining the Rules

A crucial part of any new customer complaint-management system is defining the rules that are to govern its operation. It is important to identify all likely incidents and link each one of them to an appropriate response. A useful tool for doing

this is a decision table, which will enable the organization to define precise rules for action in each case. To construct a decision table it is necessary to identify all situations (conditions). These would include product or service faults (e.g., wrong weight, minor contaminant, serious contaminant, life-threatening fault, rude salesperson, poor after-sales service) and disposition of the customer (e.g., happy, neutral, angry, threatening action). It is also necessary to agree on the appropriate response (e.g., days to respond, call on the customer, etc.).

62.3.3.2.2 Complaint Classification

Once all conditions have been identified, a complaint classification table can be constructed. The classifications are to identify a predetermined response for every situation. Table 62.10 shows five different responses denoted by the letters A, B, C, D, and E. If all of the conditions, actions, and appropriate response rules have been defined, there will be a basis for a sound and reliable procedure.

62.3.3.2.3 Appropriate Responses and Operation

Once there is agreement on the complaint classifications, the appropriate responses need to be linked to them. Table 62.11 shows the general idea: By determining the complaint classification the person dealing with the complaint can select the correct response every time. If required, another column ("else") can be added to meet any situation that does not fit A, B, C, D, or E.

TABLE 62.10
Complaint Classifications

Complaint	Customer			
Wrong weight	A	A	B	C
Minor contaminant	A	A	C	D
Serious contaminant	B	C	D	E
Life threatening	E	E	E	E
Rude salesperson	A	B	C	D
Poor service	A	B	C	D

TABLE 62.11
The Response Rules

	Customer				
Action	**A**	**B**	**C**	**D**	**E**
Days to respond	3	1	1	I	I
Standard letter	Y	N	N	N	N
Special letter	N	Y	O	N	N
Vouchers	2	3	N	N	N
Call on customer	N	O	Y	Y	Y
Retrieve product	N	O	Y	Y	Y
Recall procedures	N	N	N	O	Y
Advise QA manager	N	O	Y	Y	Y
Advise GM	N	N	O	Y	Y

62.3.3.2.4 The Responsibility Matrix

To help define who is responsible for what, one can employ a responsibility matrix. The idea is to relate people and required actions in a single table (see Table 62.12).

62.3.3.3 Recording, Monitoring, and Reporting

To turn quality problems into improvement opportunities, it is essential to record and monitor all relevant information. Depending on the size of the organization and the number of complaints, anything from a simple manual system to a sophisticated computer system may be required. For most organizations, a modest PC-based system will prove the best solution. However, irrespective of whether the system is computer-based or not, the general principles are essentially the same: The system must provide for ease of monitoring, analysis, and subsequent reporting.

62.3.3.3.1 Origins, Sources, and Causes

In an earlier section, we looked at the origin, sources, and causes of quality problems. It is important that the customer-complaint system and procedures and the staff operating them are capable of differentiating between origins, sources, and causes. If there is no such distinction, it is unlikely that the management of customer complaints will lead to process improvement and a subsequent reduction in quality failures. Of particular concern is that many organizations do not appear to appreciate the difference between common-cause and special-cause quality problems.

In organizations where the difference between common and special causes is not understood, one often finds major changes to systems and procedures because of some out-of-the-ordinary failure. Also, failures due to common-cause variability may be explained to customers as one-time problems that are unlikely to recur.

62.3.3.3.2 Recording Details

For a customer complaint system to be effective, the proper recording of all necessary details is essential. Figure 62.6 shows an example of a monthly recording of the details of origins, sources, and causes. Similarly, other information such as complaint classification and the nature of the complaint (basic, satisfier, or delighter) should be recorded. An action and improvement log should also be maintained.

62.3.3.3.3 Analysis and Reporting

If a good recording and monitoring system is in place, subsequent analysis and reporting can be tailored to fulfill particular needs. A company may settle for a simple form of analysis and reporting, in which case the task is fairly straightforward. On the other hand, it may decide to be quite sophisticated and opt to employ advanced analytical techniques and reporting methods. Whatever approach is adopted, it is crucial that the analysis and reporting have some real meaning leading to improvements in performance. The following points should be kept in mind:

More sophisticated reports: The information collected should allow one to identify possible correlations, for

TABLE 62.12
Responsibilities for Customer Complaints

	Staff Responsible				
Functions or Actions	**Customer Services Clerk**	**Customer Manager**	**Area Sales Representative**	**Dispatch Clerk**	**Etc.**
Complaint receipt	R	A	R	R	
Fill out form	R	R			
Determine classification	A	R			
Initiate response	A				
Call on customer					
Send replacement					
Etc.					

Note: R = response; A = assist.

No.	Origin				Source'	Cause	
	Design	Execution		Use	Description of source	Common	Special
		Int	Ext				
001	✓				Specification limits beyond machine quality.	✓	
002		✓			New operator		✓
003	✓				Scraps of packing material can occasionally enter the product.	✓	
004			✓		Outside contractor working on machine failed to clean grease off weighing bucket.		✓
031							
032							
Total	10	19	2	1		22	10

FIGURE 62.6 Origins, sources, and causes of complaints.

example, between (i) complaint classifications and origins; (ii) particular types of complaints such as contamination and origins; (iii) origins and causes; (iv) reductions in complaints and improvement projects. (A word of caution. When embarking on a program of more sophisticated analysis, be sure to employ a suitably numerate employee. Basic conceptual errors can make any analysis at best useless, at worst dangerous.)

Action/improvement reporting: The main source of information for this will be the action and improvement log, although information may also come from other sources such as customers and operations. Control of dates is important (e.g., date items first raised, original agreed completion date, latest agreed completion date).

Place in company reporting: Once the analysis to be done and reports to be produced have been determined, it is necessary to decide on the content of the regular monthly report. It is also necessary to decide who will be responsible for the reporting and where the report should be included.

62.3.3.4 Improving Process

Ensuring the correct customer response in every case is a crucial feature of any complaint system. This part of the system is "outward focused," which is very important to maintaining good customer relations. However, to improve performance and hence the reputation as a quality company, it is necessary to have an inward focus as well.

Customer complaints can provide valuable information about underlying weaknesses. However, the important point to note here is that what shows up as customer complaints are effects or results and that to prevent or lessen complaints we must identify and improve the underlying processes. It may be said that this statement is rather obvious, however it is a fact that many organizations do not bother to search out the processes involved with permanent improvements. It should be remembered that most management reporting (not just customer complaints) is about results with little or no appreciation of what are the relevant processes.

62.3.3.4.1 Managing Improvements

A system of recording and monitoring will identify many areas where improvements are both possible and necessary. Improvements should be managed in a structured and somewhat formal manner. Some points to remember include:

The customer complaint system, if managed correctly, provides information as a basis for improvement rather than emotive assertions such as: We always have the wrong weights; we never deliver on time; or our product is full of contaminants. The emphasis needs to be on prevention and improvement rather than culprit finding. It must be quite clear whether problems are common-cause (systems) or special-cause types. Generally speaking, improvements or a reduction in complaints come about by focusing on and improving the underlying processes. It is necessary to exercise patience. Improvement of the process will take time, but if done properly the improvement will be permanent and hence very worthwhile.

62.3.3.4.2 Identifying Processes

If it is working correctly the system will identify the process that needs to be improved, e.g., staff induction/training, design procedures, specification development, production tolerances, or process monitoring. Of particular interest are complaints that have their origin in product design and development. Design is the start of the sequence of operation, and hence any failings in design will affect all subsequent processes.

As mentioned earlier, a large number of a firm's quality failures have their origin in faulty design, although they may, at first sight, appear to have their origin in faulty execution. If products are designed and developed with problem prevention in mind, many subsequent quality failures can be avoided. Important areas to consider include (i) standardization of ingredients where appropriate, (ii) simplifying processes where possible, and (iii) designing intermediate tests into the process, including statistical process control.

Once a reasonable quantity of data has been collected, it should be possible to identify the specific processes giving rise to particular complaints, such as (i) ingredient quantity control, (ii) mixing, and (iii) cooking. Note, however, that to find the real source of the problem, considerably more investigation is usually needed. For example, complaints may be traced to the cooking process, but the real source may be insufficient operator training, out-of-date manuals, or a lack of maintenance of the temperature-control equipment.

62.3.3.5 Management Commitment

The proper management of customer complaints requires much thought and effort as well as attention to detail. In addition, considerable management support and involvement is required, especially if improvements are to be made. It really comes down to the following questions: (i) is the organization serious about the customer's expectations and the company's reputation? and (ii) is it prepared to make the effort to turn problems into opportunities? Even if not all problems can be solved, we can still benefit from some of the concepts and techniques discussed, provided the organization knows where it wants to go and ensures a consistency of signals.

62.3.3.6 Consistency Is All-Important

Consistency of signals to both customers and staff is vital if one is to succeed in reducing complaints. For example, do not allow quality policies and procedures to be overridden arbitrarily by edicts from above. Be sure to provide ongoing support for the complaint system's operation and resulting improvement projects. In order to ensure that output from the system is given due recognition, it is important to (i) check the company's current culture and practices, (ii) decide what the current shortcomings are and what changes are warranted, (iii) obtain agreement from affected managers and staff to develop a plan of action, and (iv) implement and enjoy the improvements in performance. The process of improving an organization's performance and enhancing its reputation as a quality company is all about doing many things better. Focusing on customer complaints is a good starting point.

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